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TITLE OF THESIS........ Ultrastructural Changes in Skeletal Muscle
During Early Hemorrhagic Shock. A Morphologic
and Stereologic Analysis.

UNIVERSITY........ of Alberta

DEGREE FOR WHICH THESIS WAS PRESENTED... Doctor of Philosophy

YEAR THIS DEGREE GRANTED........ 1976

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ULTRASTRUCTURAL CHANGES IN SKELETAL MUSCLE
DURING EARLY HEMORRHAGIC SHOCK.
A MORPHOLOGIC AND STEREOLOGIC ANALYSIS.

by

©

DAVID RONALD ANTONENKO

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in

EXPERIMENTAL SURGERY

DEPARTMENT OF SURGERY

EDMONTON, ALBERTA

SPRING, 1976

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled ULTRASTRUCTURAL CHANGES IN SKELETAL MUSCLE DURING EARLY HEMORRHAGIC SHOCK. A MORPHOLOGIC AND STEREOLOGIC ANALYSIS by DAVID RONALD ANTONENKO in partial fulfilment of the requirements for the degree of DOCTOR OF PHILOSOPHY in EXPERIMENTAL SURGERY.

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Date . [illegible] April 1976 . . .

To my wife Suzanne

and sons

Mark, Paul, Geoffrey and Peter

ABSTRACT

Insufficient knowledge of fluid shifts at the capillary and cellular areas during shock has resulted in considerable controversy regarding the type and volume of solutions to be used in the treatment of shock. The prime contributor to this lack of knowledge has been an inability to measure with accuracy the distribution of extracellular fluid, particularly a 'non-functional' compartment, during shock.

Skeletal muscle reflects body water distribution and is partly responsible for many of the cardiovascular changes in normal and abnormal states (Berne and Levy, 1972; Graham *et al*, 1967; Muldowney and Williams, 1963). The volume of sarcoplasmic reticulum, a subcellular organelle of skeletal muscle recently described as being 'extracellular' (Birks and Davey, 1969) approximates the volume of the undefined 'non-functional' phase of extracellular fluid.

The aim of the project was to assess quantitatively and qualitatively the response of the cell and subcellular organelles (particularly sarcoplasmic reticulum) to acute hemorrhagic shock. Quantitative assessment was obtained by morphometric analysis of electron micrographs of skeletal muscle biopsied from rats submitted to acute hemorrhagic shock.

The qualitative effects of fixatives and methods of fixation have been well described (Hayat, 1970). Our results, however,

demonstrate specific quantitative effects on muscle. Extracellular space volume measured on electron micrographs is inversely related to buffer tonicity. *In vivo* and *in vitro* methods of fixation result in the same cell, sarcoplasmic reticulum and extracellular space volumes but different mitochondrial volumes. Fixation by carpal tunnel perfusion of fixative is unreliable for quantitative analysis.

Morphologic continuity between sarcoplasmic reticulum and mitochondria is demonstrated and confirms previous reports. In addition, indications of direct continuity between mitochondria and the extracellular space have been found. Since continuity between the outer nuclear membrane and sarcoplasmic reticulum is well recognized, the endomembrane system of skeletal muscle cells (and probably most cells) is structurally and possibly functionally extracellular. This impression is confirmed when the muscle response to shock is examined.

Cell volume does not change during the pre-transfusion phase of shock. In contrast, mitochondria and particularly sarcoplasmic reticulum volumes change significantly in response to shock. A shift of mitochondria from the central to peripheral regions of the cell and from A-band to I-band may occur during shock, perhaps reflecting as yet undefined functions of this organelle. Some evidence also exists for direct correlation between extracellular space volume and mitochondrial volume changes during shock.

Extracellular space volume decreases as expected during shock. However, extracellular space volume can be equated with reported chemical estimations only if the volume of the endo-membrane system of the cell is included in the histological assessment of 'total' extracellular volume in both normal muscle and muscle from rats submitted to acute hemorrhagic shock. The 'non-functional' component of extracellular fluid is equivalent to the endomembrane system of the cell.

Consequently, swelling of subcellular organelles, specifically mitochondria and sarcoplasmic reticulum, during shock must not be equated with intracellular swelling.

ACKNOWLEDGEMENTS

Many people have helped the author in the preparation of this thesis and it is with sincere gratitude that he acknowledges the assistance of:

Dr. W.J. Gibson, for his guidance and friendship.

Mr. Gus Duchon, for technical assistance.

Dr. R.A. Macbeth, for his encouragement and patience.

Dr. J.C. Russell, for his critical comments and assistance.

Mrs. J. Ebert, patience is a virtue.

Dr. K. Kowalewski, for his kindness.

This work was supported by the Medical Research Council of Canada. The author gratefully acknowledges receipt of a Medical Research Council Fellowship from 1970-1973.

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CHAPTER I

INTRODUCTION

Shock is perhaps one of the most difficult-words to define since we still have no precise knowledge of what shock is. Well over one hundred different types of shock have been described in the literature (Table I) (Hardaway, 1968), having nothing more in common than they all can terminate in death from unknown or little known causes.

The older definitions of shock; "a rude unhinging of the machinery of life" (Gross, 1850); "....a species of functional concussion by which the influence of the brain over the organ of circulation is deranged." (Travers, 1836), have not been improved upon by more modern attempts; "....progressive vasoconstrictive oligemic anoxia" (Harkins, 1940); "....peripheral circulatory failure resulting from a discrepancy in the size of the vascular bed and the volume of the intravascular fluid" (Minot and Blalock, 1940); "....a default in the transport mechanisms of the body, generally affecting vital cells" (Rhoads, 1961).

Shock defined as "....an inadequate capillary perfusion" (Hardaway, 1968) is insufficient since there are certain conditions (septic shock) where capillary perfusion is more than adequate but failure of the organism still occurs. Shock defined as a "....stage in the pathways of circulatory deterioration which may lead to death"

(Thal, 1971) is similar to Warren's (1895) "....a momentary pause in the act of death". This latter concept embraces much that is currently acceptable, particularly the notion that shock is not an entity in itself but rather an adaptive response to life-threatening injury of varying origin and marked by temporary or permanent damage to vital organ function.

Our pre-occupation with the responses of the circulation to injury are reflected by the above definitions even though it has been repeatedly stated that the arterial blood pressure, although being one of the constant signs of shock, is "....not the essential thing let alone the cause of it" (Archibald and Maclean, 1917). Viability of the cell is the final determinant of survival or death in any response to injury.

"Inadequate blood flow to vital organs or failure of the cells of vital organs to utilize oxygen" (MacLean, 1971) covers all areas and will be accepted for the present as 'the' definition of shock.

Classification

A classification based on this definition is given in Table 2. It is simple, allowing recognition of the basic problem and suggesting appropriate therapy. It represents a modification of the classifications of MacLean (1971) and Thal (1971).

The literature on shock is overwhelming. In the following dissertation the term shock will refer only to the response of the organism to blood loss, ie, hemorrhagic shock.

Circulatory Response to Hemorrhage

The circulatory mechanisms with which the body responds to hemorrhage are constant. The degree to which they respond are governed by the rate, duration and severity of blood loss.

Following a slow continuous blood loss or an acute loss of less than 20% of total blood volume the venous return to the heart, central venous pressure, cardiac filling and cardiac output temporarily decrease. The resulting drop in pulse pressure due to a reduced systolic pressure stimulates aortic arch and carotid sinus baroreceptors which inhibit the baroreceptor center in the brain stem. This inhibition of a center which normally actively inhibits the autonomic outflow tracts produces an increase in sympathetic activity and a reduction in parasympathetic vagal tone. The latter results in a tachycardia which tends to maintain or even increase cardiac output and systolic pressure.

The autonomic nervous system's response to hemorrhage has been extensively reviewed (Chien, 1967). The increasedsympathetic activity following acute blood loss produces both venous and arterial constriction. The former actively increases flow to the central veins, the latter increases peripheral resistance. With the increase in peripheral resistance, the diastolic pressure will increase, decrease or remain constant depending on the degree to which the cardiac output falls and the heart rate increases. Regardless, the end result is a drop in capillary pressure because of precapillary constriction reducing inflow pressure. Since exchange of water across the capillary wall is directly related to capillary pressure (as well as tissue pressure and net colloid oncotic pressure) the result is a net shift of fluid from the interstitial space into the capillaries. This 'capillary refill' phenomenon may be as great as 90-120 ml per hour for the first two hours after hemorrhage (Moore, 1965). This may be sufficient to maintain an effective circulating blood volume following a 10-20% loss of blood volume. With any greater degree of blood loss where this sympathetic response and capillary refill are unable to compensate both systolic and diastolic pressure drop. A further stimulation of the sympathetic and parasympathetic nervous systems results and a vicious circle ensues resulting in an inability to maintain perfusion. At this stage decompensation is said to occur. Continuation of this cycle eventually results in death.

It is generally accepted that most of the capillary refill occurs from the interstitial space. A decrease of 5% of cell volume has been reported by Shizgal *et al* (1968) implying a contribution of cell water to capillary refill. Newton *et al* (1969) has demonstrated no change in the intracellular water content of muscle, lung or liver during shock. Although the majority of evidence favors no involvement of cell water in capillary refill, such involvement remains to be defined.

Shoemaker (1967) has divided the response to hemorrhage into early, middle and late phases. The early and middle phases are arbitrarily divided by the lowest mean arterial pressure attained following hemorrhage, the middle and late phases are separated by the point at which the arterial pressure returns to normal following treatment (Figure 1). This separation into phases has the advantage of defining areas of pathogenic mechanisms, modifiers of pathogenic mechanisms and terminal mechanisms with [illegible] pathogenic significance in the early, middle and late phases respectively.

This separation into phases on the basis of arterial pressures is also subject to error if one accepts the definition of shock outlined previously, ie, a defect in capillary perfusion. Arterial pressure is not necessarily an accurate indicator of peripheral perfusion (Desai *et al*, 1969). Frequently the arterial pressure may be normal in the very early stages of hypovolemia but be associated with extensive areas of hypoxic tissue due to severe arterial constriction. Low arterial pressures with a decreased peripheral resistance as a result of high spinal cord injury or anesthesia may be attended by normal capillary perfusion with reduced resistance. During septic shock low arterial pressures may be associated with increased capillary perfusion (Wright *et al*, 1971). Here the primary defect appears to be cellular rather than circulatory.

The use of arterial pressure to define stages of shock is traditional however, and until more accurate methods of assessing perfusion are more generally accepted and used (membrane potentials, peripheral tissue pH), the above separation into phases will be followed.

The majority of shock research deals with the late or post-transfusion phase, ignoring the fact that knowledge of mechanisms of injury in the early phase would result in prevention of complications and perhaps increased survival. This thesis will therefore deal only with the early and middle stages of shock in an attempt to define capillary and cell reaction to hemorrhage. Reference will be made to the late phase only if it has direct application to the development of the hypothesis which forms the basis of this thesis.

Fluid Therapy in Shock

The treatment of hypovolemia with intravenous fluids was first reported by Latta (1831) who infused 330 ounces of saline into a person suffering from cholera and then noted a dramatic improvement in the patient's condition. O'Shaugnessey (1832) demonstrated that the collapse associated with cholera could be treated successfully with intravenous administration of fluids containing sodium and potassium in the same concentration as existed in the diarrhea stools. However, in spite of these reports of successful treatment of shock with saline solutions, hypodermocyclysis and the "occasion enema" (Treves, 1899) continued to be the standard treatment for hypovolemia until the use of blood transfusions was accepted in the 1920's as a form of therapy for hypovolemic shock. The salutary effects of saline infusions as therapy for hypovolemia was re-emphasized by Wangensteen (1916) and fortunately ended the 'adrenal exhaustion' concept of hypovolemia suggested by Corbett (1915). This latter proposal suggested repeated doses of epinephrine for therapy of shock but resulted in a high mortality rate.

The works of Latta, O'Shaugnessey and Wangensteen were confirmed by Warren (1895), Crile (1899), Cannon and Bayless (1919), Johnson and Blalock (1931) and Beecher (1952) who proved that a reduction in circulating blood volume was the most important cause of shock due to fluid loss or trauma. Restoration of the loss of circulating blood volume with saline or blood increased survival and reduced the morbidity associated with shock. The experiences of two world wars and the Korean conflict only reinforced this therapeutic approach.

In 1960 Shires *et al* suggested that saline be used in addition to blood transfusions and in volumes greater than the calculated blood loss. He demonstrated increased survival in dogs subjected to a Wigger's shock protocol as compared to those dogs treated with either saline or blood alone. Others have since confirmed the superiority of combined saline and blood.

The concept of increased saline requirements following shock was based on measurements of fluid loss from different compartments of the extracellular space. Shires *et al* (1960, 1961, 1964), Shires (1965), Crenshaw *et al* (1962), Carrico *et al* (1963) and Mathews and Douglas (1969) demonstrated in both animal and human experiments that following hemorrhage a deficit in functional extracellular fluid existed which could not be accounted for by blood loss alone. The measured decrease in extracellular fluid volume was 18% to 41% greater than the measured blood loss (Carrico *et al*, 1966; Pluth *et al*, 1967; Mathews and Douglas, 1969). The disparate reduction in functional extracellular fluid volume was confined mainly to the extravascular component of the extracellular space and was only restored by infusion of saline solutions.

Measurement of Extracellular Fluid Volumes

The authors reporting unaccounted reductions in extracellular fluid volumes (ECFV) calculated ECFV by injecting a known quantity of

S^{35}-sulphate intravenously and then measuring the concentration of the isotope 20 minutes later. They assumed that the 20 minute concentration of the isotope reflected the ECFV accessible to sulphate since radio-sulphate had been reported to reach full equilibrium with the extracellular space within 20 minutes (Walser *et.al*, 1954). The volume of the extracellular space could then be calculated by:

$$V = \frac{Q - E}{C} \qquad (1)$$

where:

V = volume of the extracellular space,

Q = quantity of the isotope injected,

E = amount of the isotope excreted, and

C = plasma concentration of the isotope at 20 minutes.

By measuring plasma volume (PV) with radio-iodinated serum albumen (I^{131}) and red cell volume (RCV) with Cr^{51} tagged red blood cells, the extravascular component (Ve) of the extracellular space could be calculated by:

$$Ve = VS^{35} - (PV+RCV) \qquad (2)$$

The reduction in extracellular space which could not be explained on blood loss alone was reported by these authors to occur in Ve, the extravascular component of the extracellular space.

As a consequence of these studies indicating such a 'disparate reduction' in extracellular fluid volume, infusions of saline in excess of calculated losses were recommended to restore the deficits. Saline infusions equivalent to 9 times the measured blood volume loss were reported (Dillon *et al*, 1966). With the advent of the Viet Nam conflict improved facilities and longer survivals from major trauma resulted in implications that these large volumes of saline were partly responsible for the 'shock lung' syndrome. Consequently Moore and Shires (1967) called for moderation in the use of saline solutions in the treatment of shock until the effects of saline on body tissues had been more extensively investigated.

Evidence conflicting with the above concepts rapidly appeared in the literature. Criticisms of Shires' results were based mainly on the methods which he had used to measure the extracellular fluid compartments.

St. Ville and Shoemaker (1961) showed that equilibration of Cr^{51} tagged red cells was delayed during shock as a result of the reduction in flow rate in peripheral tissues. Other studies indicated that the equilibration time for S^{35}-sulphate in the normal animal exceeded 120 minutes (Cleland *et al*, 1966; Schloerb *et al*, 1967; Roth *et al*, 1967; Rothe, 1970; Vineyard and Osborne, 1967; Shizgal and Gutelius, 1967), not 20 minutes. Furthermore the equilibration time for radio-sulphate was prolonged during shock consequent to reduced flow in the periphery (Anderson *et al*, 1967; St. Ville and Shoemaker, 1961), preferential distribution of the marker in the plasma (Vineyard and Osborne, 1967) and reduced diffusion of water soluble materials in the interstitial fluid during shock (Koven *et al*, 1970; Cotton *et al*, 1970; Gallie *et al*, 1971).

Sulphate is also incorporated into metabolic pathways in the liver and gut (Dzietwiatkowski, 1949; Lowe and Roberts, 1955; Williams and Woodbury, 1971), is bound to mucopolysaccharide (Kragelund and Dyrbye, 1967) and has a slower rate of equilibration than sodium or thiocyanate (Pluth *et al*, 1967; Koven *et al*, 1970). The loss of S^{35} sulphate into cells as a result of altered cellular permeability as suggested by Moore (1965) could not be confirmed by Furneaux and Tracy (1970).

Due to these many factors the shorter equilibration times used by Shires and others reporting 'disparate' reductions in extracellular fluid volumes would result in extracellular fluid volumes which were less than the volume which actually existed. This perhaps accounts for some of the discrepancies noted by these authors.

Using longer equilibration times of over 180 minutes, multiple stage sampling and extrapolation of the resulting curve back to zero time, many of the above authors demonstrated no change in extracellular fluid which could not be explained by either dehydration prior to measurement or blood loss. Use of markers such as inulin, thiocyanate, sodium and bromide by the same authors also demonstrated no change in extracellular fluid volume during shock. Some even demonstrated an increase in extracellular fluid volume during extensive surgery (Pluth *et al*, 1967; Rothe, 1970).

A summary of extracellular fluid volume measurements during shock is shown in Table 3 and reflects the controversy over fluid shifts during shock.

In view of the initial criticisms of Shires' work, Middleton *et al* (1969) studied the dilution curve of sulphate in normal and shock situations and demonstrated three phases for the sulphate dilution curve. The first and most rapid phase requires 20 minutes for equilibration and represents dilution of the isotope in the vascular system. The second slower phase of up to 120 minutes duration represents equilibration of the isotope with the interstitial fluid space. These two phases represent the 'functional extracellular fluid' described by Shires and Carrico (1966). Both phases of equilibration are prolonged during shock for the reasons noted above.

The final phase of isotope distribution represents 15% of the total sulphate space and is thought to represent either organic binding sites (Walser *et al*, 1954; Kragelund and Dyrbye, 1967; Barratt and Walser, 1968) or as yet unidentified spaces in organs such as liver and muscle (Middleton *et al*, 1969).

Cell Response to Shock

Shires and Carrico (1966) suggested that the final 'non-functional' space for sulphate equilibration represents a shift of fluid into the cell during shock as a result of cell hypoxia and interference with cell membrane transport.

Campion *et al* (1969) used microelectrodes to measure the membrane potential (MP) of skeletal muscle cells during hemorrhagic shock. Rapid decreases in MP from -89 mv in the normal animal to -60 mv in the animal submitted to a 40% hemorrhage occurred within 15 minutes after the onset of hemorrhage. The MP paralleled the arterial pressure. They assumed that the drop in MP was on the basis of cell hypoxia and interference with energy forming processes consequent to reduced capillary flow but no metabolic studies or measurements of capillary flow were performed to confirm these assumptions. With this drop in MP they predicted on the basis of the Nernst equation a loss of potassium from the cell and an influx of sodium (and water) into the cell, thus producing cell edema.

Haljamäe (1970), using interstitial fluid aspiration with micropipettes, confirmed an increased interstitial potassium concentration very early in shock corresponding in time to the drop in membrane potential. Lemieux *et al* (1969) noted the same effect using potassium specific microelectrodes. This increased interstitial potassium concentration occurred much earlier than any change in arterial pressure or pulse suggesting an alteration of capillary flow or a direct effect on the cell of some toxin released during shock.

Structural alterations in liver and muscle cells appear to confirm this concept of an intracellular shift of fluid during late shock. Hift and Strawitz (1961), Holden *et al* (1965) and DePalma *et al* (1970) demonstrated extensive swelling of the mitochondria and sarcoplasmic reticulum following prolonged hemorrhagic shock induced using a Wigger's shock technique. These changes were presumed to occur on the basis of hypoxia due to reduced capillary flow. However, Crowell and Smith (1964) showed in rats that such ultrastructural changes were primarily due to changes in capillary flow and not hypoxia. This was also reported by Schildt (1972) who produced severe hypoxia in a hind limb by applying a tourniquet around the limb and completely occluding it for up to 4 hours. He noted swelling of the mitochondria and sarcoplasmic reticulum only after the tourniquet had been released and a reactive hyperemia with increased capillary flow and pressure had occurred. Cellular swelling during the early stages of shock which would correspond to the changes in membrane potential noted by Campion *et al* (1969) have not been reported.

Stewart and Rourke (1936) and more recently Albert *et al* (1967) and Shizgal *et al* (1968) have demonstrated a loss of cell water rather than the gain as suggested above using similar shock protocols. Rocchio *et al* (1973) noted no change in cell water in skeletal muscle following shock. He, as well as others (Dillon *et al*, 1966; Moyer *et al*, 1965; Monafo *et al*, 1971) noted deficits of up to 15% in muscle sodium during untreated prolonged shock. Slonim and Stahl (1968) and Newton *et al* (1969) noted an increase in skeletal muscle sodium in those animals which died following shock, possibly supporting Campion's suggestion of a shift of sodium into the cell and a decrease in MP. However, no increase in cell sodium occurred until the post-transfusion phase of shock, long after the decrease in MP had occurred. No increase in cell sodium was noted in those animals which survived.

The sodium content of connective tissue increases during shock (Fulton, 1970; Marty and Zweifach, 1971) as a result of adsorption of sodium onto collagen or an alteration of the extracellular protein-polysaccharide-water gel (Slonim and Stahl, 1968). This may account for at least some of the 'intracellular' sodium noted above. Whether sufficient sodium is adsorbed onto collagen to produce significant effects on the cardiovascular system or on interstitial diffusion as claimed by Koven *et al* (1970) remains to be verified in man.

A summary of reported changes in cell water and electrolytes during hemorrhage are noted in Table 4 and reflect the uncertainty surrounding this field.

It appears therefore that during late shock the cell responds with a drop in MP and swelling of the mitochondria and sarcoplasmic reticulum. Confirmation of these responses suggesting cell edema with analysis of muscle water and sodium content has not been convincing, particularly in the early and middle phases of shock.

Extracellular Space

The confusion which exists regarding changes in tissue water after hemorrhage stems basically from an inability to accurately measure the extracellular space. "Unfortunately there is currently no known substance whose volume of distribution includes only and all the extracellular fluid." (Cizek, 1968). Measurement of total body water can be performed with considerable accuracy using tritiated water. However measurement of cell water is inconsistent since it depends on accurate measurement of extracellular fluid volume.

The extracellular fluid phase is a heterogeneous collection of fluid which may be divided into four categories: (1) the vascular system, (2) the interstitial space (including lymph) which bathes the cells and is perhaps less than 0.5 microns in thickness (Cizek, 1968), (3) fluid from bone, cartilage and dense connective tissue, and (4) transcellular fluids including cerebrospinal fluid, peritoneal and pleural fluids. The distribution of the body fluid compartments is shown in Figure 2. It is the vascular space and interstitial space components of the extracellular space with which we are primarily concerned during shock.

Materials that have been used most often to measure extracellular space *in vivo* and *in vitro* include sodium, chloride, bromide, sulphate, thiosulphate, thiocyanate, sucrose, inulin, mannitol, urea and albumin. Histological methods have been used also but these are subject to many factors during sampling, embedding, sectioning, etc., as described in Methods. The extracellular space varies from 8% of body water with the higher molecular weight substances such as albumin (Hill, 1964) to over 40% with materials such as chloride (Tasker *et.al*, 1959; Johnson and Simonds, 1962). These discrepancies are evident for muscle both *in vivo* and *in vitro*. The only consistent finding has been an inverse relationship between the molecular weight of the marker used to measure the extracellular space volume and the volume measured.

Skeletal Muscle and Shock

The roles of various organs in the fluid shifts occurring during shock have been only recently investigated. These include the kidney, lung, heart, liver and intestine. The last is prominently involved late in shock in dogs and rats but minimally involved in man (Marty and Zweifach, 1971). However, skeletal muscle has been almost totally neglected in the investigation of the pathogenesis of shock with only brief coverage being given to it in even the most extensive works on shock (Ballinger, 1968; Pollock, 1966; Shoemaker, 1967; Thal, 1971). This, in spite of the fact that muscle is used almost routinely for analysis of fluid spaces and electrolyte content, and is an accurate representative of total body water and electrolyte content in normal (Graham *et al*, 1967) and pathological states (Muldowney and Williams, 1963). It provides much of the pH (Couch *et al*, 1971; Van de Water *et al*, 1972; Lemieux *et al*, 1969; Filler *et al*, 1972), electrolyte (Haljamäe and Rockert, 1967) and lactic acid (Schumer, 1968) change during shock.

Skeletal muscle represents almost 40% of total body weight and 50% of total body water (Cizek, 1968; Woodbury, 1965) and contains a capillary network that would require more than double the normal cardiac output if fully expanded (Cizek, 1968). Its circulation is very sensitive to most vasoactive materials, being responsible for many of the changes in blood pressure, peripheral resistance and other cardiovascular parameters (Ballinger, 1968; Berne and Levy, 1972). The surface pH of muscle is also a more accurate indication of tissue perfusion and cell function than the plasma parameters usually measured (Couch *et al*, 1971).

Following hemorrhage, sympathetic stimulation results in only a slight increase in vasoconstriction in muscle since its arterioles are felt to be almost maximally constricted in the normal state (Chien, 1967). Very rapidly however an arteriolar vasodilation occurs due to a systemic release of epinephrine, a local release of metabolic products such as hydrogen ion, lactic acid, adenine nucleotides and to hypoxia. Thus a reduction in muscle resistance with normal or increased flow occurs initially with compensated increases in heart rate and cardiac output (Chien and Gregersen, 1968). During decompensation capillary flow will decrease due to reduced arterial pressure.

Reductions in membrane potential (Campion *et al*, 1969), loss of cell potassium (Haljamäe and Rockert, 1967), increased interstitial pH (Couch *et al*, 1971) and structural evidence of cell edema (swollen mitochondria and sarcoplasmic reticulum) (Holden *et al*, 1965) result from decreases in capillary flow.

It becomes obvious that the role of skeletal muscle during shock is significant and does not warrant the neglect shown it by most authors. A more thorough evaluation of its response to shock is required.

Structure of Skeletal Muscle

Each skeletal muscle cell is enclosed by a cell membrane (sarcolemma) and contains the cytoplasm (sarcoplasm), contractile filaments, nuclei which are subsarcolemmal, mitochondria, endoplasmic reticulum (sarcoplasmic reticulum or SR) and glycogen granules. The cross striations from which the term striated muscle is derived reflects the darker anisotropic (A-band) and lighter isotropic (I-band) regions seen with the light microscope (Plate 1). Each I-band is bisected by a Z-line whereas each A-band is bisected by a lighter area, the H-zone. In the center of the H-zone is the M-line which represents the area of junction between the two myosin chains forming the thick filaments represented by the A-band. Thin filaments only are within the I-band. The area from the edge of the H-zone to the A-I junction is an area of overlap of thick and thin filaments. A sarcomere extends from one Z-line to another. Mitochondria, glycogen granules and SR are interposed between and around the myofibrils (Plate 2).

On cross-sections the striated appearance is absent but the relation of the thick and thin filaments is defined (Plate 1a to 1e).

The sarcoplasmic reticulum is divided into three regions each connected with the other. The terminal cisternae in the I-band are connected to central H-zone sacs by longitudinal tubules. This distinction is much less evident in rat muscle than in frog muscle.

Extending inward from the sarcolemma are complex tubular structures known as transverse tubules or t-tubules. They may extend in as funnel-like invaginations of the sarcolemma (Huxley, 1964; Page, 1968; Peachey, 1965a, 1965b) or connect with subsarcolemmal vesicles called caveolae. Longitudinal branching of T-tubules may also occur (Forssman and Girardier, 1970).

The area of contact between the T-tubules and terminal cisternae has been called the triad junction (Plate 3a) although diad to septad junctions (Plate 3b) may exist. It is at the triad junction that coupling between sarcolemmal depolarization and SR release of calcium occurs to initiate contraction. In amphibians the triad junctions occur along the Z-line but in rats they occur along the A-I junction.

A schematic representation of the ultrastructure of skeletal muscle is presented in Figure 3.

The type of connection which SR forms with the T-tubules is a subject of controversy. The area consists of membranes of SR and

T-tubule between which are regularly spaced electron dense structures (Plate 4). Franzini-Armstrong (1970, 1971) considers the triad junction only as an area of electrical coupling, the interposed electron dense structures representing protein bridges or foot processes (Fig. 4a). Others (Birks, 1965; Birks and Davey, 1969; Walker and Schrodt, 1966; Walker *et al*, 1969, 1970) propose that a direct anatomical communication represented by pores exists between the terminal cisternae and the T-tubule (Fig. 4b). Birks (1965) further states that SR is therefore an extension of the extracellular space and subject to the same parameters that affect interstitial space although probably modified.

There is a growing body of evidence to support the latter concept. If the osmolarity of the extracellular fluid is increased or decreased the resulting change in SR volume is opposite to that expected for an intracellular organelle (Birks and Davey, 1969; Davey, 1969). If cardiac muscle (which has a structure very similar to skeletal muscle) is perfused, the SR reacts as the extracellular space reacts to sudden variation in osmolarity of the perfusate (Ferrons *et al*, 1971) or to perfusion pressure (Brown *et al*, 1969).

In view of the possible extracellular character of SR it has been proposed that SR is a selective site of penetration by small molecular weight substances from the extracellular space (Birks and Davey, 1969; Dydynska and Wilkie, 1963) but not larger molecules. This would explain the inverse relationship between molecular size of an extracellular space marker and the extracellular volume measured. More recent evidence of penetration of the SR by larger molecules such as thorium dioxide (Rubio and Sperelakis, 1971) and horseradish peroxidase (Rubio and Sperelakis, 1972) adds credibility to the concept. Such penetration of SR is inversely related to molecular weight and directly related to time of contact of the molecule with the cell.

The difference between functional and total extracellular fluid volume is 15% or 4% of total body water (Douglas *et al*, 1969; Middleton *et al*, 1969; Newton *et al*, 1969). Sarcoplasmic reticulum accounts for 13% of total cell volume in amphibian muscle (Peachey, 1965b; Davey, 1969) and 7% to 18% of total muscle volume in the biceps of the muscle of the mouse (Goldspink, 1971). If one assumes that human muscle has a similar volume fraction of SR then about 5% of total body water is accounted for by this organelle in muscle alone. This is a volume equivalent to total blood volume and large enough to account for the 'non-functional' component of extracellular fluid described previously.

Sarcoplasmic reticulum has been shown to swell during shock (Hift and Strawitz, 1961; Holden *et al*, 1965; DePalma *et al*, 1970). If the diameter of SR was to increase during shock by as little as 25%, SR volume would increase by more than 50%, an increase which is equal to plasma volume. If one also assumes that SR is extracellular with respect to some species of smaller molecules as has been suggested, then relatively small changes in SR volume during shock could account for many of the discrepancies in extracellular space volume reported (Table 3).

The goal of this investigation was to assess both qualitatively and quantitatively the early response of skeletal muscle to acute hemorrhage. Knowledge of the morphologic and quantitative response of the cell and its organelles, particularly the sarcoplasmic reticulum might provide a clue to the location of many of the fluid shifts which occur during shock.

CHAPTER II

METHODS

A. ANIMAL MODEL

The best model for learning about the effects of hemorrhage as they occur in man is man himself. The clinical picture however is often complicated by uncontrollable variables and accurate definition of the effect of one variable may be clouded by the effect of others. These innumerable variables can be modified or controlled to some extent in the experimental animal such that the effect of one variable may be studied.

The use of experimental animals for studying the problems of shock has not however eliminated the need for precise definition of the experimental model. Variables considered in animals include species and strain differences, age, sex, anesthetic, seasonal variation, environmental temperature, diet, adaptation to injury, and the method of hemorrhage.

Two categories of hemorrhagic shock models have been used: (a) those in which a fixed volume of blood was withdrawn, regardless of changes in arterial pressure; and (b) those in which enough blood was withdrawn to reduce the mean arterial pressure to a predetermined level.

The latter technique was most commonly used on the assumption that "the reduction of blood pressure at least roughly approximated the decrease of blood supply to the peripheral tissues" (Selkurt and Rothe, 1961).

In this reservoir model blood was allowed to flow from the arterial system of the animal into a reservoir. The mean arterial pressure was determined by the height of the reservoir above the heart. After a variable period of time blood began to return to the animal spontaneously ('uptake phenomenon'). The remainder of the shed blood in the reservoir was re-infused into the animal and the physiological changes or the effects of therapy were studied. Arbitrary variables included the rate of hemorrhage, the degree and length of hypotension, the percentage uptake and the rate of re-infusion of shed blood.

This technique, popularized by Wiggers (1950) and modified by Fine and Seligman (1943), Lamson and de Turk (1945), Lillehei (1957) and others, led essentially to a study of the irreversible changes depending on the variables listed above. Its popularity was enjoyed even though "in clinical surgery, medicine and pediatrics, the term irreversible shock should not be used because it is inappropriate both biologically and therapeutically" (Moore, 1961). The portal and splanchnic congestion with its attendant hemorrhagic intestine has been accepted as typical pathological change in shock induced by this model. Recently it has been shown that these changes were a result of the experimental design, having occurred only after re-infusion of shed blood following prolonged hypoxia (Marty and Zweifach, 1971; Swan and Nelson, 1971; Fronek and Zweifach, 1971).

The reservoir model "....may be useful for comparison of tolerance to hypotension...." (Bloor *et al*, 1958) but it eliminated the study of the hemodynamic homeostatic mechanisms which are an integral part of the clinical picture of hemorrhagic shock in man (Ehrlich *et al*, 1969). Furthermore there is no clinical situation that parallels the reservoir model.

The acute hemorrhage model (Swan, 1965; Swan and Nelson, 1971) or its modification (Ehrlich *et al*, 1969; Desai *et al*, 1969) had the advantage of resembling the clinical condition of shock. Although the response to bleeding a specified volume varies with each animal and is only partly predictable (Ehrlich *et al*, 1969), the model allowed the animal to maintain unimpaired homeostatic mechanisms which could be studied.

B. ANESTHESIA

Barbiturates, in spite of their popularity, are a poor choice for studies involving the response of the microcirculation in skeletal muscle to hemorrhage since they inhibit most of the normal homeostatic responses to hemorrhage.

Barbiturates have been shown to increase the blood volume of the splanchnic circulation (Aarseth and Piene, 1972; Rieke and Everett, 1967) and reduce the blood volume of the pulmonary and skeletal muscle circulations prior to hemorrhage (Aarseth and Piene, 1972). They depress spontaneous respiratory activity (Soma, 1971), inhibit oxidative phosphorylation (Harris *et al*, 1971) and reduce sympathetic nervous system activity which is essential for normal response to acute hemorrhage.

Methoxyflurane (Penthrane, Abbott Laboratories) is more suited for study of an animal's response to acute hemorrhage. It does not significantly depress the sympathetic nervous system (Skovsted and Price, 1969) and has no effect on total limb blood flow (Black and McArdle, 1965). However, the effects of anesthetics on regional flow have not been defined. The adverse effect of myocardial depression which is common to all anesthetics (Bagwell and Woods, 1962; Hudon *et al*, 1963; Walker *et al*, 1962) and renal toxicity (Crandell *et al*, 1966; Elkington *et al*, 1968; McIntyre and Russel, 1971) are dose dependent and may be minimized by shallow planes of anesthesia (Skovsted and Price, 1969). Use of methoxyflurane resulted in an easily controllable plane of anesthesia and a stable normal arterial pressure in contrast to pentobarbital (Fig. 5). Normal fluctuations in mean arterial pressure which are due to respiratory activity were clearly visible on the arterial pressure recording in a methoxyflurane anesthetized rat but not in a pentobarbital anesthetized rat.

C. GENERAL METHODS

Male Wistar rats (Woodlyn Farms, Guelph, Ontario) weighing 300-490 grams were kept in self cleaning cages in air conditioned rooms at 22.5°C for at least two weeks before experiments were performed. They were fed a standard pellet diet (Purina) with water *ad libitum* until 18-24 hours before study when they were given only water. No overlap between series of rats used was allowed thus ensuring homogeneity in each series with respect to weight, age, diet, environment and season.

Method of Anesthesia

Induction of anesthesia in the rat was performed by placing the rat's head into a glass beaker containing a 4x4 surgical sponge soaked with methoxyflurane. There was usually no struggle by the rat because they appear to like the odor of methoxyflurane and will burrow their noses into the sponge. The rat's neck, abdomen and groin were shaved after it was weighed.

Continued anesthesia was maintained according to the nose cone method described originally by Hagen and Hagen (1964). Tracheotomy or other means of artificial pulmonary support was unnecessary. 100% oxygen bubbled through methoxyflurane in a bottle flows into a polyethylene nose cone fitted over the rat's head (Fig. 6). The rate of oxygen flow controlled the plane of anesthesia by directly varying the concentration of methoxyflurane delivered to the rat. Reducing the flow of oxygen resulted in an increased concentration of methoxyflurane. Increasing oxygen flow would decrease methoxyflurane concentration but increase the rate of induction. This latter effect is achieved by rapidly equilibrating the inspired concentration of methoxyflurane with the concentration of methoxyflurane in the vaporizer (Soma, 1971).

Arterial Pressure Recording

The left carotid artery was exposed with the aid of a 5X magnifying glass and cannulated with PE 100 Intramedic polyethylene cannula tubing with care being taken to avoid the vagus nerve. The cannula was connected to a Statham pressure transducer (Model P-23AA) with a three-way stopcock which was also used for the withdrawal of blood required to produce the predetermined reduction in mean arterial blood pressure. A Gilson Medical Electronics 4 channel physiological recorder, calibrated before and at least once during each experiment, recorded the arterial pressure at a chart speed of 6 cm/minute. The total time required from the onset of anesthesia to initial pressure recording was usually 10-15 minutes.

All tubing used was filled with heparinized saline (100 units sodium heparin/ml 0.9% saline) prior to cannulation of the carotid artery, care being taken to remove all air bubbles from the cannula. Back-flow of blood into the cannula was flushed out with 0.1 ml of heparinized saline every 5 minutes during the control period and after each blood withdrawal. The only visible effect of the flush on the arterial pressure was a very brief increase in pulse pressure but no change in mean arterial pressure (Fig. 7).

Method of Hemorrhage

The sequence of hemorrhage and biopsy as shown in Figure 8 was followed in all experiments. The technique of hemorrhage followed the method described by Ehrlich *et al* (1969).

Following a control period of 15 minutes during which the arterial pressure was allowed to stabilize following cannulation, the mean arterial pressure was reduced by a series of rapid blood withdrawals until 40% of the estimated blood volume (50 ml/kg; Sréter, 1964) was removed. A withdrawal of 1.5-2.0 ml of blood usually decreased the mean arterial pressure 15-20 mm Hg within 3-4 minutes. The final mean arterial pressure was 30-40 mm Hg, attained within 25 minutes (18-25 minutes). This degree of hemorrhage was associated with a mortality of 50% if no treatment was given (Swan, 1965).

Muscle Biopsy

Muscle biopsies were taken at the completion of the control period and at each step during reduction of the mean arterial pressure, recognizing that each rat will respond differently to the same volume of hemorrhage (Swan and Nelson, 1971; Fronek and Zweifach, 1971; Ehrlich *et al*, 1969). The rat was sacrificed after the final biopsy was taken.

Two different muscles were examined. Lumbrical muscles were used because of their peripheral location and their excellent suitability for *in situ* fixation for electron microscopy (Landon, 1966). The adductor brevis in the thigh was used for its proximal location in the thigh and its shape which is ideal for serial biopsy in the same animal.

D. ELECTRON MICROSCOPY

Fixation for Electron Microscopy

The quality of tissue preservation is dependent not only on the characteristics of the fixative used but also on the method of applying the fixative to the tissue. In most cases *in situ* fixation has been preferred to *in vitro* fixation if the situation permits.

Two methods of *in situ* fixation are available; intravascular perfusion and micro-injection. The latter involves the injection of fixative directly into the tissue. Intravascular fixation, although more expensive and difficult to use, has several advantages over *in vitro* or immersion fixation. The rate and depth of penetration of the fixative are increased (Hayat, 1970), the artifactual effects of hypertonic and hypotonic solutions are reduced (Maunsbach, 1966) and autolytic changes in the tissues are minimized, particularly in tissues such as myocardium which are very sensitive to hypoxia (Trump and Erickson, 1965). Whereas immersion fixation preserves the superficial layers of tissue best, intravascular fixation preserves most areas of the tissue rapidly and uniformly as a result of shorter diffusion distances for the fixative. Muscle in particular is more accurately preserved since it remains attached and extended during fixation (Hayat, 1970). One disadvantage of intravascular fixation is prevention of serial biopsies in the same animal since the animal dies once fixation has started.

Carpal tunnel fixation (Merrillees, 1960) is claimed to provide excellent fixation of the tissue *in situ*. This involves injection of small volumes of fixative into the carpal tunnel of the hind limb of the rat. The fixative diffuses along the lumbrical muscle sheaths, preserving the muscles *in situ*.

All three methods of fixation, intravascular perfusion, carpal tunnel perfusion and immersion fixation, were used in the study.

Buffers and Fixatives

All solutions were made from reagent grade chemicals and double distilled, de-ionized water. Osmolarities of the solutions were measured by freezing point depression using either an Advanced Instruments Osmometer or a Precision Instruments Osmette. The pH of all solutions were measured with a Fisher Accumet pH Meter, Model 210.

"Comparatively little attention has been given to the effect of specific ionic composition of buffers on the preservation of fine structure." (Hayat, 1970). Although modifying effects of different buffers are less apparent if the tissue is initially fixed with glutaraldehyde *in situ*, this has not been true for *in vitro* fixed tissue.

Bicarbonate buffer has several advantages over other buffers. It is more efficient than phosphate buffer in preserving cellular integrity (Cone, 1968). Sectioning of epon embedded tissue is usually easier than with phosphate buffer and it does not produce distorted endoplasmic reticulum (Hayat, 1970).

Solutions

Krebs' Ringer Solution

	mM/L	gm/L
NaCl	137.0	8.000
KCl	5.0	0.373
$NaHCO_3$	12.0	1.008
$CaCl_2$	2.0	0.294
$NaH_2PO_4 \cdot H_2O$	0.5	0.069
Glucose	5.0	1.000

Osmolarity = 296 mOsm/L

Although it has been generally accepted that the fixative should be slightly hypertonic for muscle (Hayat, 1970; Davey, 1973), Cone and Denton (1971) stated that the buffer osmolarity should be 0.6 M if minimum osmotic effects of the fixative on the tissue were to be avoided. Since this has a direct bearing on this study, the effects of both hypotonic buffered glutaraldehyde and isotonic buffered glutaraldehyde on muscle during shock were studied.

The total osmolarity of the hypotonic buffered glutaraldehyde was slightly hyperosmolar with a 1% concentration of glutaraldehyde (322 mOsm/L). For the other fixative, 2.5% glutaraldehyde was used since a concentration of 1.7-3.5% glutaraldehyde has been recommended for muscle (Fahimi et Drochmans, 1965).

1% Glutaraldehyde

6.5 parts Krebs' ringer } 190 mOsm/L
3.3 parts distilled water }
0.2 parts glutaraldehyde (50%)

Osmolarity = 322 mOsm/L

2.5% Glutaraldehyde

9.5 parts Krebs' ringer
0.5 parts 50% glutaraldehyde

Osmolarity = 585 mOsm/L

The final osmolarity of the Kreb's ringer was 286 mOsm/L.

1% Osmium Tetroxide

5.0 ml Krebs' ringer
5.0 ml distilled water
0.1 gm osmium tetroxide

Intravascular Perfusion Fixation

The technique for intravascular fixation followed that described by Ovalle (1971) and Landon (1966). After the mean arterial blood pressure had been reduced by hemorrhage to a predetermined pressure the rat's abdomen was opened through a midline incision and the right common iliac artery cannulated with PE 100 Intramedic polyethylene tubing filled with heparinized saline. Krebs' buffered glutaraldehyde at room temperature (to minimize the vascular effects of temperature) was infused at a constant rate of 2.29 ml/minute using a Harvard constant volume infusion pump. A total of 100 ml of fixative was infused before the muscles were biopsied and placed in the same fixative at 4°C.

Good perfusion was accompanied by rapid blanching of muscle which slowly developed a light yellow color during perfusion and by the appearance of glutaraldehyde exuding from the tip of an amputated toe. The inferior vena cava was cut to allow unobstructed drainage of fixative from the hind limb.

Since perfusion pressure during fixation may affect the quality of fixation (Gil, 1971; Weibel, 1963), the pressure was measured and found to stabilize within 3 minutes at 145 mm Hg regardless of the mean arterial pressure at which fixation was started (Fig. 9). A similar perfusion pressure was found to give excellent preservation of lung (Gil, 1971; Gil and Weibel, 1971).

In vitro Fixation

In each rat similar muscles in the hind limb opposite to the perfused limb were used for either carpal tunnel fixation (Merrillees,

1960) or immersion fixation. If the ischemia time for any of the biopsies exceeded 5 minutes, the rat was excluded from the study since even this short time may produce subtle changes in volume of some intracellular organelles particularly mitochondria.

The muscles were placed in glutaraldehyde at 4°C for 24-72 hours before embedding.

Embedding

After initial fixation with glutaraldehyde the muscle was washed with three changes of Krebs' buffer; post-fixed with 1% osmium tetroxide for 1-2 hours, the time varying with the size of the muscle sample (usually 1 mm x 1 mm x 5 mm); dehydrated with serial concentractions of ethanol followed by two washings with propylene oxide; and then placed in 50% propylene oxide-50% Epon 812 resin mixture for 2-3 hours.

Each muscle was then cut into two pieces and placed in molds containing Epon 812 resin, oriented in such a way that both transverse and longitudinal sections could be cut from the same muscle (Fig. 10). The following day they were placed in an oven at 60°C for a further 48 hours and then allowed to cure at room temperature for one week before sectioning.

Sectioning and Staining

The tissue was sectioned with a Dupont diamond knife in a Sorvall Porter-Blum MT-2 ultra-microtome. Grey or silver-grey sections were cut from the middle third of a muscle fasciculus and picked up on 200 thin mesh or 150 regular mesh copper grids. Use of smaller mesh grids resulted in too few cells present on one grid square to allow accurate calculation of extracellular space.

Before sectioning the initial 0.5 mm of muscle was trimmed with a razor blade to remove any areas damaged prior to fixation. Before a grid of sections was picked up 20-30 sections were trimmed from the block with the diamond knife. 20-30 thick sections (gold or purple) were removed again before a further one or two grids were picked up. This sequence permitted examination of different areas of muscle. Areas with large gaps between fasciculi or with large vessels or nerves visible on the block face were avoided. Sections from the center of the fasciculus allowed a more consistent comparison between samples with respect to interstitial space and capillary changes since it is the effects of capillary perfusion with which we are primarily concerned.

The sections were stained with lead citrate and uranyl acetate (Reynolds, 1963) before examination.

Electron Microscopy

All tissues were examined with a JEOL JEM 7A electron microscope at an accelerating voltage of 80 kv with objective aperatures of 30 to 50 microns and an attached liquid nitrogen cooling trap.

A carbon grating with a mesh of 28,000 lines per inch for low magnifications and 54,840 lines/inch for high magnifications was used to calibrate the magnifications with each series of experiments. Although calibration is not strictly necessary if point counting techniques are applied (Weibel, 1969) such calibration is essential for the measurement of the average interfilament distance at high magnifications. The error associated with the calibration of magnification was less than 2%.

Photographs of representative sections were taken using either Kodak Projector Slide Plates (3x4 inches) or estar thick base Kodak electron microscope film, No. 4489, of the same size. Measurements of a carbon grating and of the width of the emulsion after developing demonstrated that film and plates could be used interchangeably. Both were developed with Kodak D-19 developer full strength for plates and 50% for film; fixed with Gaf Acid Fixer and Hardener in two baths of 5 and 10 minutes and then dried in room air after washing in tap water for at least one hour.

Final enlargements of the micrographs for stereological measurements were obtained using a Durst Laborator 138-S enlarger. Rather than print the micrographs before measurement the micrographs were projected with the enlarger directly onto a counting grid. For closer viewing or for publication, the micrographs were printed on Kodak Kodabromide F-4 or F-5 contrast paper on Ectamatic SC photographic papers with Kodak contrast filters. Kodak Dektol developer was used for the prints.

E. HISTOLOGICAL MEASUREMENTS

Various problems are associated with measuring volume fractions of tissue components from histological sections, particularly electron microscope sections. An ultrathin section randomly cuts through various components of the tissue resulting in profiles of the components. Subjective interpretation of these profiles is usually possible but may be erroneous. For example, a circular profile may be derived from spherical, elliptical, conical or cylindrical structures. Also the size of a profile is not necessarily representative of the size of the structure from which it arose since various factors such as the angle of sectioning and the thickness of the section may influence the interpretation of the profile.

A frequently used method of measuring the volume fraction of a tissue component involves cutting out and then weighing from photomicrographs the profiles of each component. This technique and polar planimetry (both methods of areal analysis) are "rather cumbersome and may be affected by considerable error depending on the shape and size of the profiles" (Weibel, 1969). Observer bias may also increase the variance associated with areal analysis. Hilliard and Cahn (1961) also demonstrated that areal analysis (involving the use of a planimeter or other means of assessing area) is associated with a greater variance than systematic point counting procedures, which will be described later. Methods of measuring diameters of profiles such as for sarcoplasmic reticulum (Peachey, 1965b; Birks and Davey, 1969) are open to subjective interpretation as to the direction in which the diameter will be measured and furthermore such measurements rely on some assumption as to the shape of the organelle represented by the profile.

The method we chose to use is reliable, free from bias (providing certain precautions are taken), relatively easy to use and, as noted above, associated with less variance than other methods of assessing volume. No assumptions as to shape of a structure need to be made.

Stereology has been defined as the "interpretation of three-dimensional structure from two-dimensional images (Underwood, 1970).

The fundamental relationship of stereology (DeLesse, 1847) states that "the planimetric fraction of a section occupied by sections of a given component corresponds to the fraction of the tissue volume occupied by this component". This means that the volume fraction (density) VVi of a component i in the tissue can be estimated by measuring the area fraction AAi of a random section occupied by transection of i:

$$VVi = AAi \quad (3)$$

A similar relationship has been derived for lineal analysis of the volume fraction (Rosiwal, 1898). However the simplest and most efficient method (Hennig, 1959; Weibel, 1969) of measuring the volume fraction of a component involves superimposing a regular point lattice on the section (Fig. 11) and counting the points which lie on transections of the component (Glagoleff, 1933):

$$VVi = PPi \quad (4)$$

where PPi is the fraction of points included in the profile of the component i.

Therefore,

$$VVi = AAi = PPi \quad (5)$$

The mathematical validity of these relationships has been repeatedly demonstrated. In addition, as discussed previously, volume fractions (densities) obtained by systematic point counting are affected by smaller overall error than those obtained by areal or lineal analysis (Hilliard and Cahn, 1961).

With point counting the volume fraction of each component i is given generally by

$$VVi = \frac{Pi}{PT} = PPi \qquad (6)$$

where PT is the number of points on the counting grid and Pi is the number of points overlying the component i.

A simple regular square lattice grid (Fig. 12) is used for estimating volumes of relatively large organelles within the tissue. In the case of components whose volume fractions or profiles may be small, a double lattice grid system is used (Fig. 13) (Weibel, 1969; Elias *et al.*, 1971). In this case the volume fraction VVi of a component i is given by:

$$VVi = \frac{Pi}{g^2 x PT} \qquad (7)$$

where g is the ratio of small to coarse lines in the double lattice grid system.

For our experiment we used a double lattice grid with g equal to 3, d equal to 1.2 cm and a equal to 0.4 cm (Fig. 13). Coarse points were used to calculate extracellular space volume at low magnification and mitochondrial volume at high magnification. Fine points were used for measuring capillary volumes and sarcoplasmic reticulum volumes at low and high magnifications respectively.

To minimize subjective interpretation of the data, the following protocol was followed:

1. In the series where vascular perfusion of fixative was used,

only one rat could be studied at each level of hypotension. The rats were randominized before study as to the mean arterial pressure which would be attained before fixation was started. Where two methods of fixation were compared in the same rat the technique of fixation was maintained for each limb (right or left) in each successive rat.

2. During post-fixation with osmium tetroxide the muscle samples were coded by a second person not involved with this study. This code was not available until all calculations had been completed for each series.

3. Four blocks of muscle were obtained from each sample. Two of these blocks containing muscle oriented optimally for sectioning were then chosen. Sections from different parts of the muscle were obtained as described previously.

4. For calculation of interstitial space volume fraction and capillary volume fraction, micrographs were taken from two different quadrants on each of two to three grids. The micrographs were positioned such that the lower edges of the micrograph were adjacent to the lower edges of the grid square. Consequently a minimum of four sections per muscle were examined from the total number of sections obtained from the blocks.

5. For calculation of the volume fractions of different organelles and interfilament distances, micrographs were taken from the edge of a cell adjacent to a capillary and in the center of the cell, in both A-band and I-band regions on cross-sections of the cells. This enabled assessment of the total cell response to shock.

6. Point counting was carried out by a technician who was an unbiased observer.

The rats were divided into the following groups:

Group 1: Six rats were subjected to 1% glutaraldehyde in hypotonic buffer fixation at various degrees of hypotension following acute hemorrhage. Two methods of fixation were used, vascular perfusion using a Harvard constant volume infusion pump and carpal tunnel perfusion. The latter technique was later discarded since variables such as elevated tissue pressure which have significant effects on measurement of capillary and interstitial space volumes could not be controlled. This will be discussed later.

Since Starling's hypothesis applies during vascular perfusion fixation this study might allow examination not only of the effect of fixation on the muscle but also the effect of a hypotonic perfusate on muscle during shock, perhaps analogous to infusion of hyptonic solutions during rescusitation of shock patients.

Group 2: Six rats were used to compare the effects of vascular perfusion and immersion of isotonic buffered glutaraldehyde fixatives on muscle during shock.

Group 3: The pressure during infusion of the fixative has a specific effect on the tissue (Gil, 1971). Three rats were studied to determine the effect of normal pump pressure and perfusion pressure equal to the mean arterial pressure at which perfusion is started.

Group 4: Since results obtained from the above experiments could be explained on the basis of individual animal variation, two rats were used to allow serial biopsy of adductor muscles during similar degrees of hypotension resulting from acute hemorrhage. Tissues were fixed *in vitro* using 2.5% glutaraldehyde in isotonic buffer and compared with similar tissues in the other series. The results from the two rats were added together after ensuring that no statistical difference existed for any of the components measured in the two rats.

The following measurements were made in all muscles.

VI = volume fraction of interstitial space
VC = volume fraction of total capillary
VL = volume fraction of lumen of the capillary
VM = volume fraction of mitochondria
VSR = volume fraction of sarcoplasmic reticulum
IFD = average distance between the thick filaments

In addition, VM, VSR and IFD within each sarcomere were assessed at the edge of the cell and in the center of the cell to obtain a complete analysis.

Therefore; within the A-band:

AN = volume fraction adjacent to the sarcolemma
AA = volume fraction in the cell center
AT = average volume fraction in the A-band

Within the I-band:

IN = volume fraction adjacent to the sarcolemma
IA = volume fraction in the cell center
IT = volume fraction in the I-band

For each sarcomere:

SN = volume fraction adjacent to the sarcolemma

SA = volume fraction in the cell center

ST = volume fraction in the cell

All values are expressed as fractions of tissue volume. Where applicable, organelle volumes can be calculated as fraction of cell volume by dividing organelle volume by I-(VI+VC).

F. STATISTICS

All results are expressed as the mean ($\bar{x}$) for a specific number of observations n for each animal. The method of determining n for morphometric sampling will be described later. Tests for the significance of the difference between two means were performed using a standard routine for an unpaired t-test available on an Olivetti 101 calculator.

DeHoff (1967) has shown that the number of measurements n required to obtain an accuracy of y percent (where twice the standard deviation equals y) can be obtained by:

$$n = \left(\frac{200}{y} \times \frac{\sigma x}{\bar{x}} \right)^2 \qquad (8)$$

where σx is the standard deviation of single observations and $\bar{x}$ is the sample mean value. If n is too large for time or monetary reasons, the level of accuracy required can be reduced and fewer measurements would be required.

The number of animals used for each experiment does not influence this calculation. However the number of animals used in each experiment could influence comparison between experiments in direct relationship to the number of animals in each group. However, as will be shown, the time and cost of using increasing numbers of animals combined with morphometric techniques becomes almost prohibitive.

Table 5 illustrates the time required to assess each muscle for each experiment. For example, for 6 adductor muscle samples in one experiment, 36 micrographs per sample or 216 micrographs per experiment were examined. The minimum number of coarse and fine points counted for each micrograph, section, specimen, and experimental group is also listed. The time required for complete analysis of each step after photography is complete is shown in the lower section of the table. These figures do not include the time for fixation and embedding, sectioning and statistical analysis for each experimental group. By fixing and embedding samples from several animals the time related to this process could be cut by one-half. However, for a series of six rats this still would require 90 hours. The total time required for each experiment would therefore be 120 hours.

If the number of animals per experiment (ie, per arterial pressure level) were increased by a factor of 3 the time for each experiment would increase by as much as 240 hours. For the four series of animals planned for this project this would amount to an additional total time of 960 hours. This working time combined with limited availability of various instruments, particularly the electron microscope, could extend the time of the project by at least 1 to 1 1/2 years and increase the cost considerably.

The variance V_x of an experimental mean can be estimated by:

$$V_x = \frac{S_0^2}{n_0} + \frac{S_1^2}{n_0 n_1} + \frac{S_2^2}{n_0 n_1 n_2} + \frac{S_3^2}{n_0 n_1 n_2 n_3} \qquad (9)$$

where S_0 is the variance of a group of n_0 animals, S_1 is the variance of the number of sections n_1, S_2 is the variance of the number of micrographs in n_2 per section and S_3 is the variance of the number of points n_3 scanned per counting field.

As shown above, increasing the number of animals per experiment was prohibited by cost and time. Therefore the variance was improved by increasing the number of sections (n_1), and micrographs (n_2) examined.

Using formula 8 the number of micrographs to be examined was estimated from analysis of a small sample of muscle. The number of micrographs required to achieve a specific accuracy (±2 S.D.) for each parameter measured is shown in Table 5. Due to the reduced level of accuracy accepted $P<0.10$ were accepted as significant; $P<0.05$ very significant and $P<0.01$ highly significant. Due to the greater accuracy associated with measurement of IFD significance limits for this parameter were accepted as $P<0.05$.

CHAPTER III

METHOD OF FIXATION

Carpal Tunnel Fixation

Lumbrical muscles fixed with 1% glutaraldehyde injected into the carpal tunnel (Merrillees, 1960) yielded an excellent quality of fixation in the superficial fibers of the muscle with minimal evidence of extraction of cell components (Plate 5) and excellent preservation of cell organelles (Plate 6). However the center of the muscle showed significant evidence of cell edema with extraction and swelling of mitochondria (Plate 7).

The differences in appearance between the peripheral and central areas of the cell may reflect a combined effect of external compression of venous and arterial supply to muscle, alteration of normal transcapillary exchange and relatively slow diffusion of fixative through the tissue.

When the fixative was injected into the carpal tunnel an uncontrolled increase in pressure occurred within the fascial compartment surrounding the lumbricals. The initial effect may have been an obstruction of the venous flow from the muscle and subsequently increased capillary pressure. An increased interstitial fluid volume would be expected based on Starling's hypothesis governing transcapillary fluid exchange. With higher compartment pressures, arterial obstruction may occur causing cell hypoxia, acidosis and then cell edema. This could occur within 15-20 minutes of hypoxia (Firket and Beaumariage, 1971). Since glutaraldehyde requires up to 30 minutes to penetrate 1 mm of tissue (Hayat, 1970) acidosis and subsequent cell edema could occur in the center of lumbricals prior to fixation.

If the above hypothetical sequence of events actually occurred during fixation by carpal tunnel injection we could expect (1) elevated capillary pressure, (2) increased interstitial fluid volume and (3) cell edema.

Table 7 compares the capillary, interstitial space, cell, mitochondrial and sarcoplasmic reticulum volumes for lumbricals fixed by carpal tunnel injection of 1% glutaraldehyde to lumbricals fixed by arterial perfusion of the same fixative but in the opposite hind limb of the rat.

Lumbricals fixed by carpal tunnel injection have a greater total capillary volume ($P<0.10$) consistent with the postulated effects of venous obstruction. A greater VI exists for lumbricals fixed by carpal tunnel perfusion but it is not significantly greater ($P>0.01$). A significantly greater mitochondrial volume ($P<0.05$) occurs which would be the expected result of cell acidosis and hypoxia. However cell water content ($P<0.01$) and sarcoplasmic reticulum volume ($P<0.01$) are decreased opposite to what might be expected from the differences in mitochondrial volume.

The differences between the two methods of fixation are similar for VM and capillary volume. There is an inverse relationship between cell volume and mitochondrial volume. Since both lumbricals are from the same animal and have been preserved with the same fixative it is unlikely to be a specific effect of the fixative. One may postulate that one limb of the animal is different from the other but this is highly unlikely. The relationships of mitochondria with the cell and extracellular environments will be considered in much more detail later.

Although carpal tunnel fixation of lumbricals yields excellent preservation of superficial fibers it was decided not to use this method for further experiments for three reasons; (1) the uneven fixation between the peripheral and central areas of the muscle; (2) interference with venous and perhaps arterial supply to the muscle during fixation as the pressure in the fascial compartment increases with injection of fixative and (3) the profound effect which this pressure increase could have on the response of the vessels to hemorrhage. An initial series utilizing carpal tunnel fixation during shock was performed. Not only were the results erratic but no correlation could be obtained for the response of the tissue fixed by carpal tunnel injection with other methods of fixation.

Arterial Perfusion versus Immersion Fixation

The interstitial space volume of muscle fixed by arterial perfusion of 2.5% glutaraldehyde is 31% greater for lumbricals ($P<0.10$) but 1.8% less for adductor brevis ($P>0.10$) in comparison to the same muscles fixed by immersion in the same fixative (Table 8). The slight difference for lumbricals in the two methods of fixation partly confirm the finding in brain tissue of a smaller interstitial space volume in tissue fixed by immersion (Sumi, 1969).

Sumi (1969) felt that differences in intercellular spaces between *in vivo* perfused and *in vitro* fixed tissue were a result of alterations in capillary pressure during fixation. Gil (1971) showed that Starling's hypothesis governing transcapillary fluid exchange is unaltered during fixation of the lung with glutaraldehyde. Therefore a greater capillary pressure during pump perfusion could account for greater interstitial space volume in muscle fixed by this method.

As shown in Table 8 however no significant difference in total capillary volumes (VC) or net capillary pressures (VL) exists between the two methods of fixation. Therefore on this preliminary assessment differences in interstitial fluid volume between the two methods of fixation are not related to differences in capillary pressure.

The diffusion of some materials such as oxygen on the arteriole side of the circulation may be significant (Berne and Levy, 1972). Increased arteriolar pressure during arterial fixation and shift of fluid into the interstitial space across the arterioles during perfusion of the fixative might account for the slight difference in VI between the two methods of fixation.

The pump pressure during arterial perfusion (Fig. 9) very rapidly reaches a maximum of 145 mm Hg. This suggests rapid fixation of the vessels or a sudden vasoconstriction as the hyperosmolar fixative enters the larger vessels. Since VC and VL are the same it is unlikely that these factors have a significant effect on the volume fractions of the tissue components measured at the capillary level. However some trans-arterial shift of fluid during or before fixation remained a possibility.

In order to assess this possibility a comparison between constant pump pressure (145 mm Hg) and perfusion of fixative by lower gravity pressure was made. In a normal rat with a mean arterial pressure of 100 mm Hg, gravity perfusion of one hind limb was performed at a pressure of 100 mm Hg. At the same time the opposite hind limb was perfused with the pump as described in Methods. In a rat which had been hemorrhaged, both methods of perfusion were again used except the gravity pressure was now 70 mm Hg, equal to the mean arterial pressure. The results are shown in Tables 9 and 10 for lumbrical muscle and Tables 11 and 12 for adductor muscle. A comparison of perfusion methods was attempted for 50 mm Hg arterial pressure in three additional rats. However in all three rats gravity perfusion at this pressure resulted in inadequate flow of fixative and poor fixation.

The results do not confirm the concept of trans-arterial shifts of fluids during fixation. VI, VC and VL are not significantly different for the two methods of perfusion fixation. Therefore neither capillary pressure nor arterial pressure differences can account for the different interstitial space volumes between muscle fixed by perfusion and immersion.

There are however significant differences in the effect of the two types of perfusion on cell and cell organelle volumes.

VM is the same in all areas of the cell for adductor and lumbrical muscle fixed by both pump and gravity perfusion. Cell volume (IFD) is significantly greater in both muscles fixed by pump perfusion at normal pressures ($P<0.01$) and lumbrical muscle fixed at 70 mm Hg arterial pressure. IFD is significantly greater ($P<0.01$) in adductors fixed by gravity perfusion at 70 mm Hg (Table 11). VSR in adductor muscle (Table 12) is significantly greater in muscle fixed by pump perfusion ($P<0.05$), similar to the different IFD. However, in adductor muscle fixed at 70 mm Hg, VSR in the A-band is less in gravity perfused muscle ($P<0.10$) which is opposite to the IFD difference.

The different effects of the two methods of fixative perfusion on cell volume and cell organelle volume do not follow any predictable pattern. Whether the differences observed relate to the method of fixation or the effect of hemorrhage remain to be determined by future study.

The Effect of Fixation - Tonicity

The histological studies of Schultz and Karlsson (1965), Karlsson and Schultz (1965), Johnson and Roots (1967) and Patel *et al* (1971) only confirm the general impression that "the exact nature and dimension of the intercellular space remains in doubt" (Hayat, 1970).

Schultz and Karlsson (1965), and Sumi (1969) noted that the extracellular space increased in brain tissue as the tonicity of the fixative increased. This effect has also been noted in skeletal muscle (Fahimi et Drochmans, 1965). The reduction in extracellular space with progressively hypotonic fixatives apparently results from a compression of the interstitial space as the cells swell (Fahimi et Drochmans, 1965; Hayat, 1970). Bone and Denton (1971) analysed the effect of fixative tonicity on teleost scales and noted that closer approximation of the normal cell-extracellular space relationships are achieved if the tonicity of the fixative is only 60% of the tonicity of the solutions with which the tissue is normally in contact. However, in a later publication (Bone and Ryan, 1972) they stated that "the tonicity of the vehicle must be close to the osmolarity of the normal external solutions". Although hypertonic fixatives may alter the size of the extracellular compartment, they also result in better quality of fixation than hypotonic fixatives (Hayat, 1970).

The contribution of glutaraldehyde to the total osmolarity of the fixative can be safely ignored in most tissues providing low concentrations of the fixative are used (Hayat, 1970). Confirming this, Goldspink *et al* (1973) showed in mouse skeletal muscle that "the osmolarity of the fixative apparently had no effect on fiber size". A concentration of 1.7% to 3.5% glutaraldehyde yields the best quality of fixation for skeletal muscle (Fahimi et Drochmans, 1965) although concentrations as low as 0.5% have been used with good results (Hayat, 1970).

Because of the discrepancies between the above authors on the effect of tonicity on tissues, the effects of hypotonic and isotonic buffered glutaraldehyde on the quality of fixation and on the volumes of tissue components was examined.

Both muscles fixed with hypotonic buffered 1% glutaraldehyde show some separation of myofibrils and considerable extraction of mitochondria (Plates 8, 9), probably a result of low concentrations of glutaraldehyde (Hayat, 1970). Muscles fixed by immersion (Plates 10, 11) or arterial perfusion with 2.5% glutaraldehyde (Plates 12, 13) are well preserved with minimal evidence of cell edema or extraction of cell components.

The relationship between fixative tonicity and extracellular space volume is shown in Table 13 for lumbrical and adductor brevis muscles. It is assumed again that the concentration of the fixative with respect to total osmolarity is minimal (Hayat, 1970). The interstitial space volume is 30% less in lumbricals ($P>0.10$) and 39% less in adductors ($P>0.10$) fixed with hypotonic buffered glutaraldehyde as compared with isotonic buffered glutaraldehyde. This relationship holds whether individual animals or groups of animals are compared. This non-significant relationship between VI and fixative tonicity contradicts the previous reports (Shultz and Karlsson, 1965; Sumi, 1969; Fahimi et Drochmans, 1965).

In a short experiment to further test the effect of buffer tonicity on extracellular space, a single muscle biopsy was divided into three portions. These were fixed in 2.5% glutaraldehyde buffered with 0.5N, N and 2N phosphate bicarbonate buffer. The results (Table 14) confirm the positive relationship between interstitial space volume and tonicity, confirming the findings in brain (Shultz and Karlsson, 1965; Sumi, 1969) and muscle (Fahimi et Drochmans, 1965). Muscle fixed using a hypertonic buffer (2.0N) has a VI which is significantly greater than muscle fixed using a hypotonic buffer $(P<0.01)$. Muscle fixed using isotonic buffered glutaraldehyde has a VI which is slightly greater than the VI for muscle fixed by hypotonic buffered glutaraldehyde $(P>0.10)$. Consequently a significant positive relationship between buffer tonicity and extracellular space volume does exist.

CHAPTER VI

NORMAL MUSCLE

Numerous factors can influence the estimation of the volumes of each tissue and cell component. These factors include animal weight and age, the number and type of muscle fiber within each muscle, the concentration of the fixative, the tonicity of the buffer and the method of fixation.

Animal Variation

When radioisotope dilutions are used to measure body spaces an inverse relationship between body weight and extracellular space volume can be demonstrated for adult rats (Fernandez *et al*, 1966) and infant rats (Jelinek, 1961). However, age, diet and exercise can significantly alter these relationships (Simpson and Spears, 1970). Thus, for any experiment weight, age, diet, exercise and factors influencing each must be "selected in a range as narrow as possible to avoid artificial differences or an extreme dispersion of results" (Fernandez *et al*, 1966). Accordingly, for all the experiments in this project, the above factors were maintained as uniform as possible with the exception of exercise which could not be controlled.

No significant relationship between animal weight and extracellular space volume could be shown for either *in vivo* or *in vitro* fixed muscle. Furthermore correlation between animal weight and cell volume could not be found. Since cell volume contributes 80-90% of tissue volume such a relationship might be expected. Failure to show correlation between the above parameters probably relates to failure to measure 'lean body weight' in the rats since fat content in laboratory rats is often large, variable and difficult to control.

Tasker *et al* (1959) showed that not only do small muscles such as lumbricals have a greater extracellular space volume than larger muscles such as the adductor brevis, but they also show a greater variation in ECS volume when measured by either chemical or histological methods. This is illustrated in Table 15. The results represent the mean values obtained from three rats fixed by arterial perfusion and two rats fixed by immersion. The values were only grouped after t-test evaluation demonstrated no significant differences between animals.

The interstitial space volume VI for adductor muscle is significantly less than the VI for lumbricals whether fixed *in vivo* ($P<0.10$) or *in vitro* ($P<0.01$). Since no significant difference exists between muscle fixed by arterial perfusion and immersion, the individual values were grouped. The interstitial fluid volume is 18.21%±3.83% ($\bar{x}\pm$SEM) for lumbricals and 7.82±1.42% ($\bar{x}\pm$SEM) for adductors. The difference between these means is again significant ($P<0.05$). These findings confirm the findings by Tasker *et al* (1959).

In contrast, cell volume (Table 16) does not vary with the method of fixation and is the same for both muscles. The values expressed in Table 16 were evaluated in the same manner as the values in Table 15.

We can conclude therefore that cell volumes are constant regardless of muscle type. Extracellular space volumes are less for large muscles such as adductors.

Fiber Types

Mammalian muscles are composed of at least three different fiber types: red, white and intermediate. Excellent reviews of the anatomy and physiology of each type are available (Peachey, 1965a; Padykula and Gauthier, 1967; Hess, 1970; Sandow, 1970; Close, 1972).

Many morphological and histochemical criteria have been used to distinguish fiber type: (1) fiber diameter, (2) mitochondria content, (3) size and shape of myofibrils, (4) ultrastructure of the sarcomere, and (5) form of the sarcoplasmic reticulum.

Red fibers generally have a small diameter and are rich in mitochondria relative to the myofibrillar mass. The mitochondria have tightly packed cristae, may form chains running longitudinally among the myofibrils and often have branches encompassing the myofibrils at the I-band.

White fibers have a large diameter and contain few mitochondria which have few cristae and are associated with the I-band.

The intermediate fibers are intermediate in diameter and mitochondrial content. The subsarcolemmal collections of mitochondria typical of red fibers are less conspicuous.

"It is generally true however that all regions of a muscle, whether white or red to the eye, are actually heterogeneous with respect to fiber type" (Padykula and Gauthier, 1967). For many large muscles the ratio of red to white fibers in each muscle is close to one (Edstrom and Nystrom, 1970; Ariano et al., 1973).

Because all muscles areheterogeneous (Edstrom and Nystrom, 1969; Ogata and Murata, 1969; Padykula and Gauthier, 1967) no attempt was made in this study to obtain representative samples of each fiber type for each muscle in the proportions that have been outlined, (Streter, 1964; Close, 1972). Although the relative proportions of fiber types should be determined by actual count for any experiment, the time required for this would be prohibitive and is outside the scope of this thesis. Certainly when muscles from separate animals are compared, failure to perform this analysis may result in some error because of different proportions of fibers which may be inadvertently counted for each muscle. However, as noted previously, the ratio of red to white fibers is constant in the larger muscles such as the adductor brevis and approaches one (Padykula and Gauthier, 1967). Also the systematic random sampling procedure used in this study, as explained in Methods, should minimize, but not exclude, the error related to sampling of cell types.

Table 17 outlines cell volume and cell organelle volumes for five muscles from the hind limb of a rat in which the hind limb had been perfused with 1% glutaraldehyde.

The lack of relationship between cell volume and fiber type is well illustrated. Extensor digitorum longus (EDL) which has predominantly white fibers (Shafiq et al., 1966) has a significantly smaller cell volume than adductor brevis ($P < 0.01$) or gastrocnemius

($P < 0.05$) which have significant populations of red fibers. The cell volumes for lumbrical and adductor muscle are the same, agreeing with the finding for muscle fixed with 2.5% glutaraldehyde.

Muscles with a mixed population of fibers (adductor brevis, gastrocnemius) have a mitochondrial volume which is intermediate between predominantly red fibers (Soleus) and predominantly white fibers (EDL and lumbricals), an expected difference. Sarcoplasmic reticulum volume is not different for four muscle types whether related to tissue volume, total volume within the cell, or volumes in the A and I bands. The exception is lumbrical muscle which has a significantly greater SR volume ($P < 0.01$) than any of the other four muscles examined.

We may conclude therefore that in contrast to published reports; (1) cell volume cannot be relied upon to accurately reflect cell type, (2) sarcoplasmic reticulum volume is constant in its cell distribution for most skeletal muscle cells with the exception of lumbrical muscle and does not demonstrate the variation previously described for the different cell types (Close, 1972), (3) mitochondrial volume more accurately reflects cell type but the distribution of mitochondria within the cell does not differ for the various cell types.

CHAPTER V

THE ENDOMEMBRANE SYSTEM - NORMAL MUSCLE

Results

A detailed description of the ultrastructure of skeletal muscle cells has been presented in the Introduction. No attempt has been made to describe in detail or quantitate differences between red, white and intermediate muscle fibers. Excellent reviews on the structure of muscle and the different cell types are available (Hess, 1970; Close, 1972; Sandow, 1970).

In all the muscle cells examined, irrespective of cell type, close relationships between sarcoplasmic reticulum and mitochondria were noted. These relationships were particularly noted on transverse sections of the I-band, less frequently evident in the A-band. Longitudinal sections do not exhibit such relationships.

Direct membrane continuity between sarcoplasmic reticulum and the outer mitochondrial membrane are not infrequent (Plate 14). Direct continuity between two mitochondria by long (Plate 15) or short (Plate 16) tubular connections were occasionally evident. The length and multiplicity of these connections make it unlikely that they are "post-division tubules", residuals from mitochondrial fission processes (Swift, 1965) or artifacts of sectioning. The absolute frequency of such connections per sarcomere was not determined.

Very close associations between the terminal cisternae of the sarcoplasmic reticulum, mitochondria and the t-tubules were also frequently seen (Plate 17). However no direct membrane continuity was observed between mitochondria and t-tubules or between SR and t-tubules, the latter being suggested by Birks and Davey (1969). On rare occasions very close associations between SR and the cell membrane were seen in the A-band but not in the I-band. Plate 18 illustrates possible luminal continuity between a component of SR in the M-line area of the A-band and the cell membrane. The lumen of the SR component appears to communicate directly with the extracellular space. However, other interpretations are possible. This may be a sub-sarcolemmal vesicle or a part of a longitudinal extension of the t-system (Forssman and Girardier, 1970) and not a segment of SR. Furthermore, the impression of direct continuity of the extracellular space and the SR component may also be explained by membrane overlap due to the angle of sectioning.

On rare occasions direct continuity between the outer mitochondrial membrane and the sarcolemma with a pore-like communication of the intermembranous space of the mitochondrion with the extracellular space was observed (Plate 19). The pore measures 40 nm in diameter. In addition to normal mitochondria communicating with the extracellular space, degenerating mitochondria were occasionally visible immediately adjacent to or even penetrating the sarcolemma (Plate 20).

Discussion

The frequency with which mitochondria and SR are connected suggests that this is a normal occurrence in skeletal muscle and not artifact. The concept of "collision complexes" (Swift, 1965) to explain these connections in other tissues has been presented. The rare finding of direct continuity between mitochondria and the cell membrane suggests that it is either extrusion of structurally or biochemically abnormal mitochondria from the cell or that such connections are normal, the frequency perhaps relating to the metabolic activity of the cell. The latter implies that mitochondria have direct continuity with the extracellular space. Extrusion of mitochondria which are abnormal (Plate 20) contradicts the accepted mechanism of lysosomal destruction of such mitochondria.

Connections between mitochondria and endoplasmic reticulum have been reported for both mammalian and non-mammalian tissues (Bracker and Grove, 1971; Morré *et al*, 1971; Franke and Kartenbeck, 1971; Raine *et al*, 1971; Szollosi and Hunter, 1973; Walker and Schrodt, 1966; Bowman, 1967; Norberg, 1972; Ruby *et al*, 1969; Rubio and Sperelakis, 1971, 1972).

Five types of intermembrane associations between mitochondria and endoplasmic reticulum have been reported (Bracker and Grove, 1971): (1) close physical associations without contact or continuity between adjacent membranes; (2) contact or apposition of adjacent membranes without clear membrane continuity; (3) thread like continuity linking adjacent membranes; (4) direct membrane continuity but without luminal continuity between the connected compartments; (5) clear line membrane continuity accompanied by luminal continuity so that adjacent compartments are continuous in every respect.

In our observations we have been able to confirm all five associations. Plates 17 and 14 show examples of types 2 and 4 respectively. Plate 16 shows type 5 luminal continuity. Certainly types 1 and 3 could be explained purely on the basis of the angle of sectioning and the superimposition of one membrane onto or close to the other during photography. Bracker makes no mention of this. Tilt-stage attachments for electron-microscopes will be very useful in clearly defining these associations.

In addition to the structural evidence for continuity between the endomembrane system and mitochondria, Bracker and Grove (1971) presented convincing evidence for structural and biochemical similarities of the outer mitochondrial membrane and the endoplasmic reticulum (ER) membranes, metabolic interactions between mitochondria and the ER as well as functional implications of this relationship. The inner mitochondrial membrane has different staining properties, different biochemical composition and, consequently, different function. One may therefore look upon the mitochondrial matrix and inner membrane as representing a separate organelle within the endomembrane system.

The infrequent observation of direct continuity between the outer mitochondrial membrane and the sarcolemma suggests direct communication of the intermembranous compartment of the mitochondria with the extracellular space. Since the sarcoplasmic reticulum is continuous with the mitochondria and other components of the endomembrane system, then the endomembrane system including mitochondria is continuous with the extracellular space. Therefore the response of these systems to hemorrhage should be distinct from any intracellular response, but similar to changes in the extracellular environment. The small size (40 nm) of the pore-like communication of the extracellular space with the endomembrane space (if representative) suggests that only small molecular weight substances would be readily accessible to the endomembrane system, perhaps accounting for the paucity of reports of accessibility of larger molecules such as horseradish peroxidase and thorium dioxide. It is possible that the communication between SR and the extracellular space is via this endomembrane system and not via the t-tubule, terminal cisternae junction as has been suggested (Birks and Davey, 1969).

CHAPTER VI

CAPILLARIES

A. RESULTS

Normal Capillaries

Each skeletal muscle cell is surrounded by two to five capillaries which vary in size and shape. They may be rounded (Plate 21), collapsed (Plate 22) or partially collapsed (Plate 23). The general features of the capillaries are similar regardless of the method of fixation or osmolarity of the fixative. The number of vesicles in the cytoplasm of the endothelial cells and the size of the interendothelial clefts appear the same but measurements to confirm this impression were not made.

Not infrequently folds of endothelial cytoplasm project into the lumen of the capillary, often extending across most of the width of the lumen (Plate 24). Large vesicles, some in different stages of formation (Plate 25), may be present, frequently at the base of these cytoplasmic projections. The lumen of the vesicles do not contain any structural features distinguishable from plasma. In none of the capillaries is there any indication of direct continuity between the lumen of the capillary and the interstitial space. This was also true for capillaries at the lowest arterial pressures.

With the exception of lumbrical muscles fixed with carpal tunnel perfusion, the total volume and the lumen volume of capillaries in lumbrical and adductor muscles do not differ significantly (Table 18). The higher total capillary volume ($P<0.10$) in lumbricals fixed by carpal tunnel perfusion of the fixative may be related to a general increase in muscle water which occurs when the hypotonic buffer preceeds the glutaraldehyde into the muscle (Hayat, 1970) or due to venous obstruction as discussed previously. The latter is most likely since cell volume in lumbrical fixed by carpal tunnel perfusion of 1% glutaraldehyde is less ($P<0.01$) than for lumbrical fixed by arterial perfusion of 1% glutaraldehyde (Table 16).

The similarity between the volumes of capillaries preserved with intravascular perfusion of fixatives and capillaries preserved by immersion using fixative containing isosmotic buffer suggest that both method of fixation are reliable. These results agree with most authors (Maunsbach, 1966; Hayat, 1970; Davey, 1973) but disagree with Bone and Denton (1971) who suggested the use of 0.6 N buffer for minimum osmotic effect of the fixative.

Changes During Shock

When the mean arterial pressure decreases to 80 mm Hg most capillaries resemble the collapsed capillaries of normal muscle (Plate 26) with cytoplasmic projections from endothelial cells again present. The size of the interendothelial clefts and the number of vesicles do not appear different from normal capillaries.

In all muscles fixed with 2.5% glutaraldehyde, there is a significant decrease in both VC and VL following the initial hemorrhage The total capillary volumes (VC) decrease in lumbrical muscles by

67% (Fig. 14) and 64% (Fig. 15) and decrease in adductor muscles by 48% (Fig. 16) and 60% (Fig. 17). The lumen volumes decreased by 43% (Fig. 16) and 88% (Fig. 17) in adductors. The decreases in lumen volume were 80% (Fig. 14) and 53% (Fig. 15) for lumbricals. All decreases are significant as shown in the respective figures.

The decrease in total capillary volume is greater than the decrease which can be attributed to lumen volume alone. For example, in Figure 16, VL decreases from 1.43%±0.10% ($\bar{x}\pm SE$) to 0.74%±0.10% ($P<0.01$) at 80 mm Hg arterial pressure. Simultaneously, VC decreases from 0.69%±0.07% to 0.39%±0.10% ($P<0.10$). Therefore VC decreases by 0.69% but VL only decreases 0.30%. Water loss from endothelial cytoplasm is possible. The difference between total and lumen capillary volume change may also come from a reduction in vesicle water content since the total number of vesicles does not appear different from normal.

In both lumbrical and adductor muscles fixed with 2.5% glutaraldehyde total capillary volume increases to normal as the mean arterial pressure is reduced to 60 mm Hg. With further reductions in mean arterial pressure to 45 mm Hg capillary volume remains normal except for adductor muscle in series 4 (Fig. 17) where VC and VL both decrease ($P<0.10$).

At 45 mm Hg mean arterial pressure, the general structure of the capillaries does not differ from normal. Again there are no differences in the interendothelial clefts but there is an apparent reduction in the number and size of the vesicles in the endothelial cells (Plate 27).

The patterns of response of total and lumen volumes in tissues fixed by carpal tunnel perfusion or arterial perfusion of 1% glutaraldehyde is different from the pattern in muscles fixed with 2.5% glutaraldehyde. At 80 mm Hg mean arterial pressure total (VC) and lumen (VL) capillary volumes have not changed significantly after an initial increase in VL (P<0.10) (Fig. 18). The remaining values are normal. The biphasic response noted in the other series fixed with 2.5% glutaraldehyde is not seen. Total capillary volume and lumen volume do not change in lumbricals fixed by intravascular perfusion of 1% glutaraldehyde (Fig. 19), except for VC at 60 mm Hg (P<0.10). This pattern is also different from the pattern for muscle fixed by 2.5% glutaraldehyde where VC and VL decrease significantly. The increase in total capillary volume (P<0.10) with no change in lumen volume at 60 mm Hg may result from edema of endothelial cells since the number and size of vesicles do not appear different from normal. This cellular edema could result from the perfusion of a hypo-osmolar buffer which can exert an osmotic effect after the muscles have been fixed with glutaraldehyde (Hayat, 1970). The continued elevation of total capillary volume in lumbricals fixed by intravascular perfusion of 1% glutaraldehyde contrasts the changes in total capillary volume in all other muscles studied as the mean arterial pressure is reduced and may be related to this cell edema.

All the above measurements were made in different animals at different mean arterial pressures. One may argue that the pattern of response of capillary volume described previously is due to animal variation. A study was therefore conducted in which two rats were subjected to the same method of hemorrhage. Serial biopsies were taken in each rat at mean arterial pressures similar to other series, and the muscles fixed by immersion in 2.5% glutaraldehyde using the same procedure as in the previous series. Values obtained for capillary volume changes were averaged at the same mean arterial pressures after a t-test comparison of VC and VL from the two animals demonstrated no significant difference between the two animals. Adductor muscles only were studied since they, and not lumbrical muscles, are particularly suited for serial biopsies.

Figure 20 illustrates the total (VC) and lumen (VL) volumes for the two rats. The pattern of capillary response to hemorrhage is the same in this series as the pattern for adductor muscles fixed by 2.5% glutaraldehyde in the previous series (Fig. 16, 17). Following a reduction of 67% in total and lumen capillary volume at 80 mm Hg mean arterial pressure ($P<0.05$) the volumes of both capillary components return to normal levels at 70 mm Hg. Subsequently, VC and VL both remain normal as the arterial pressure decreases to 30 mm Hg.

The discrepancy between total capillary volume change and lumen volume change is again present. At 80 mm Hg VC decreases by 66% or 0.84% of muscle volume ($P<0.05$) but VL decreases by 67% or 0.59% of muscle volume ($P<0.05$). When the total volume increases to normal at 75 mm Hg lumen volume remains decreased ($P<0.05$). Therefore since the capillaries do not differ structurally from normal capillaries, one must again assume that the discrepancies are related to either changes in vesicle volume or endothelial volume.

8. DISCUSSION

The pattern of response of total and lumen volumes of capillaries is the same whether studied in the same or different animals. The responses are not influenced by seasonal variations since they were the same at all times of the year. After an initial decrease to less than 50% of normal, total and lumen volumes return to normal as the arterial pressure decreases. At the lowest arterial pressures studied both total and lumen volumes of the capillaries were normal or slightly decreased.

No structural changes developed in the capillaries as the mean arterial pressure decreased which could explain the increased capillary permeability which has been reported for the late stages of hemorrhagic shock (Appelgren, 1972).

Cytoplasmic extensions of endothelial cells (Plates 21, 24, 25) project into the lumens of normal capillaries and capillaries subjected to decreased arterial pressures following acute hemorrhage. There structure and frequency of occurrence do not appear different from normal capillaries. Although their presence has been reported previously for brain capillaries (Schultz and Karlsson, 1972), their function is unknown.

The rate of movement of fluid across the capillary membrane is directly proportional to the area of capillary membrane available for filtration (Berne and Levy, 1972). Therefore, the projections may represent a means of increasing the filtration rate by increasing capillary surface area. This would have particular value in tissues such as muscle where a substantial portion of tissue has a reduced flow at any given moment. Hence, opening or closing of capillaries need not be invoked to explain changes in filtration rates during shock (Chien, 1967). An increase or decrease in the cytoplasmic extensions could significantly alter the surface area and transcapillary filtration. Alternatively, the extensions could represent oblique sections through branching areas of the capillary (Fig. 21). However, this is unlikely. These projects are observed frequently whereas capillary branchings occur every 600 µm. Section thickness averages 50 nm. Consequently the probability of sectioning through such a branch is 1 in 3000 sections with 4 capillaries per cell each branching once in 600 µm.

Capillary diffusion capacity and net capillary pressure are approximated by the total and lumen volume fractions of capillaries respectively (Hammersen, 1970). When the mean arterial pressure decreases to 80 mm Hg, the decrease in net capillary pressure (VL) will result in a shift of fluid from the interstitial space into the vascular system on the basis of Starling's hypothesis. This reduction in net capillary pressure may result from; (1) a decrease in venous pressure following constriction of major veins induced by increased sympathetic nervous system activity in association with a decreased circulating blood volume, (2) arteriole constriction resulting in a greater pressure drop across the arterioles, or (3) a combination of the two mechanisms. The reduction in capillary pressure is more likely due to decreased venous pressure since arterioles in rat muscle (and human muscle) are near maximal constriction at normal arterial pressures (Chien, 1967), and venous pressure has a greater influence on capillary pressure (Berne and Levy, 1972).

Venous constriction continues until late in the hypotensive period (Chien, 1967). The return to normal of capillary diffusion capacity and net capillary pressure at 60-70 mm Hg mean arterial pressure is related to a reactive hyperemia as precapillary resistance decreases. The basis for this reactive hyperemia is a subject of controversy (Chien, 1967; Haddy and Scott, 1968) but includes the effects of hypoxia, hydrogen ion, adenine nucleotides, potassium ions and various other local metabolic products. The reduction in net capillary pressure at 45 mm Hg mean arterial pressure (Fig. 17) is not always evident but may be related to the low mean arterial pressure. Irrespective of the mechanism of the reduction, the net effect is a shift of fluid from the interstitial space into the vascular system on the basis of Starling's hypothesis.

According to Poiseulle's law, the flow, Q, through a cylinder varies inversely as the resistance in the cylinder, R, and directly as the net pressure drop, Pi - Po, along the length of the cylinder where Pi equals inflow pressure and Po equals the outflow pressure. The net pressure drop along the capillaries may be approximated by the net capillary pressures, Pc, which as noted above is reflected in the lumen volume fraction of the capillary. The resistance across the capillary bed (Rc) is inversely proportional to the fourth power of the radius of the capillaries when they are considered to be in parallel. However, the resistance can also be approximated by the size of the lumen of the capillaries. Capillary flow, Qc, can therefore be roughly approximated by:

$$Qc = \frac{Pc}{Rc} \qquad (10)$$

On this basis, there is a drastic reduction in muscle blood flow when the mean arterial pressure decreases to 80 mm Hg. This is followed by an increase in blood flow to normal with arteriole dilatation at 60-70 mm Hg, and a subsequent reduction as the arterial pressure is reduced below 50 mm Hg. At 30 mm Hg the capillary blood flow is only slightly decreased due to decreased Pc.

This pattern of flow in skeletal muscle during hemorrhagic hypotension is similar to that reported by other authors (Bull, 1964; Chien and Gregersen, 1968; Chien, 1969; Shoemaker, 1970, 1972). Although this histological method accurately reflects changes in capillary pressure, resistance and flow as reported in the literature, it is much more time consuming than other methods such as the Xenon clearance method and therefore could not be used consistently except as an auxiliary tool for the study of capillary dynamics.

CHAPTER VII

CELL WATER

Huxley (1953) showed from x-ray diffraction studies that the *in vivo* lattice spacing of relaxed vertebrate muscle was inversely proportional to the square root of the sarcomere length. With contraction the spacing between thick filaments also changes in inverse relation to sarcomere length (April et al., 1971). It is possible that the degree of contraction might vary between animals and within the same animal regardless of the method of fixation or biopsy technique because of changing ion and osmotic properties of the extracellular fluid during shock. It was felt that fixing the tissue without restraints would result in a more accurate representation of the cellular response to shock. Since sarcomere length was not maintained at a constant value, we elected to also measure the unit cell volume (Ks) which reflects the osmotic activity of the cell and is constant irrespective of the state of contraction (April et al., 1971).

All muscle blocks initially sectioned transversely for measurement of lattice spacing were re-oriented and sectioned longitudinally. Sarcomere length (Ls) was measured at a final magnification of 10,000 with a minimum of five measurements of Ls made from each of five different sections of a sample.

The unit cell area is the cross-sectional area of the filament lattice which is exclusive to one thick filament (Figure 22). The unit cell volume is defined as the volume exclusive to one thick filament. The unit cell area (A) is obtained by:

$$A = IFD^2 \cdot \sin 60° \qquad (11)$$

The unit cell volume (Ks) is obtained by:

$$Ks = A \cdot Ls \qquad (12)$$

By substituting equation 11 for A, Ks can then be calculated by:

$$Ks = IFD^2 \cdot \sin 60° \cdot Ls \qquad (13)$$

where IFD is the center to center distance between thick filaments and Ls is the sarcomere length. The lattice spacing of IFD previously estimated from transverse sections was used. IFD · sin 60° is equivalent to the $\bar{1}$, $\bar{0}$, lattice plane measured by x-ray diffraction (April *et al.*, 1971).

The lattice parameters for cells from various hind limb muscles of a rat are shown in Table 19 for muscles fixed with 1% glutaraldehyde and in Table 20 for muscle fixed with 2.5% glutaraldehyde. The IFD for all the muscles is greater than the IFD for frog sartorius muscle (Huxley *et al.*, 1963; Davey, 1973) but less than the spacing for crayfish muscle (April *et al.*, 1971). The differences probably relate to species differences and degree of contraction. In mammals the ratio of thin to thick filaments is 3:1, in the crayfish is 6:1 and in the frog is 2:1. One may expect therefore a greater overall interfilament spacing in the crayfish and rat to allow for sliding of thin over thick filaments during contraction and also for differences in the water content of the cells. In Table 20 measurements were grouped after ensuring that no significant differences existed between muscles.

The interfilament distances in lumbrical and adductor muscle fixed with 1% glutaraldehyde do not differ significantly (Table 19). The same is true for the same muscles fixed with 2.5% glutaraldehyde (Table 20) whether they are fixed by immersion or arterial perfusion of fixative. With both fixatives, the spacing is very constant, varying by less than 5%. There is also no significant difference in interfilament spacing between the two methods of fixation (arterial perfusion and immersion) (Table 20) for each muscle. This implies the same effect on cell volume for each fixative whether fixation is *in situ* or *in vitro*.

However, the interfilament distances for lumbricals and adductors fixed with 1% glutaraldehyde (Table 19) are significantly less ($P<0.01$) than the same muscles fixed with 2.5% glutaraldehyde. This is opposite to what is expected from the inverse relationship between buffer tonicity and myofilament spacing previously reported (Davey, 1973; April *et al.*, 1972).

Sarcomere length Ls is significantly less in both muscles fixed by 1% glutaraldehyde ($P<0.01$). Also, Ls for lumbricals fixed by arterial perfusion of 2.5% glutaraldehyde is significantly less ($P<0.05$) than Ls for lumbricals fixed by immersion (Table 20). The opposite relationship exists for adductor muscles ($P<0.01$).

A. DISCUSSION

Lattice Spacing

The discrepancies for IFD between muscle fixed with 1% and

2.5% glutaraldehyde may be explained, in retrospect, on the tonicity of the solutions used during fixation. Davey (1973) showed recently that if muscles are fixed with solutions having a tonicity which is greater than the tonicity of the solution in which the muscle was initially incubated (in this case extracellular fluid) swelling of cells would occur. This is true for tissues cut after fixation with acrolein but before osmium tetroxide fixation. If we assume that a similar effect might occur in glutaraldehyde fixed muscle, then preservation with the hypotonic fixative would result initially in cell swelling. However the muscle samples were cut and subjected to washing with slightly hypertonic buffer (Millonig phosphate, 330 mOsm/liter) before fixation with osmium tetroxide. This washing would have the effect of decreasing the lattice spacing. Whether or not this is sufficient to reduce it below the initial spacing after the hypotonic fixative is open to question.

Tissues preserved with isotonic buffered fixative and washed with the same buffer had a constant interfilament spacing regardless of method of fixation (Table 20). This only confirms the statement that "fixation of tissues exposed to modifications of normal physiological solutions should be performed using the same modified solutions as fixative vehicle" (Davey, 1973).

Consequently, all experiments with the exception of the first employing 1% glutaraldehyde, each step during fixation was carried out using the same buffer as both vehicle and wash solutions.

Effect of Compression

Compression during sectioning could produce significant distortion of the filament structure such that measurement of IFD in each of the three lattice planes might be inaccurate (Carlsen *et al.*, 1961). Because of this possibility, Davey (1973) oriented his tissue such that the line of sectioning could always be evident. The lattice spacing was measured in a plane parallel to the knife edge so that minimal distortion in measurement would occur. April *et al.* (1971) on the other hand argued that measuring the lattice spacing in all three lattice planes would minimize the effects of compression.

We must agree with April. When the lattice spacing was measured in each of the lattice planes there was less than a 5% difference in each of the measurements. Only in those sections with visible compression marks was there a greater variation and those sections were not used for final measurements of lattice spacing. Furthermore, measurements of lattice spacing from sections cut on different days, but from the same block, a condition which might produce variable compression because of changes in temperature, etc. (Hayat, 1970), were no different. Therefore, during this study, all measurements were made in each of the three lattice planes and averaged to give one value for IFD.

Sarcomere Length

The significantly different sarcomere lengths for lumbricals and adductor muscle fixed *in situ* by arterial perfusion of 2.5% glutaraldehyde most likely reflects the characteristics of each muscle.

There are also significant differences in sarcomere length for various hind-limb muscles fixed *in situ* with 1% glutaraldehyde. Consequently sarcomere length may be specific for each muscle type.

The smaller sarcomere length in adductor fixed by immersion in 2.5% glutaraldehyde probably reflects the severe degree of contraction which occurs when the muscle is cut before fixation. Lumbricals fixed *in vitro* however have a significantly greater Ls than *in situ* fixed lumbrical muscle. This may be due to active stretching of the fiber as it is dissected from the carpal tunnel structures. Since the muscle is not transected, contraction would not occur to the degree seen with adductor muscle. These effects of biopsy before fixation on sarcomere length suggest that the muscle should be biopsied and maintained at its resting length to enable valid comparisons between different muscle types.

B. CONCLUSIONS

1. Isotonic fixatives have no significant effects on interfilament distance providing the same buffer is used for all steps during fixation.

2. Sarcomere lengths for muscles fixed *in vitro* without control of the degree of stretch are not predictable and do not compare to the sarcomere lengths for muscles fixed *in situ*.

3. Adductor muscle has a significantly greater sarcomere length than lumbrical muscle when fixed *in situ*.

4. Sarcomere length may be characteristic for each muscle type.

CHAPTER VIII

CELL WATER CHANGES DURING SHOCK

A. RESULTS

The interfilament distances or lattice spacing which reflect fiber volumes (April *et al.*, 1971), the unit cell volumes representing cell osmotic activity and sarcomere length, are shown in Figures 23 to 28 for each of the series of experiments I to VI respectively. An attempt was made during each experiment to perform the biopsies at the same arterial pressures and time intervals for each animal, but this was not always possible because of slight variation in each animal's response to the hemorrhage. However, all experiments were completed within 30 minutes from the onset of hemorrhage, most within 20 minutes, making the time and pressure differences for each biopsy minimal and comparisons valid.

Interfilament Distance

The interfilament distance changes significantly as the arterial pressure decreases in all the muscles except for muscle in series III and IV. With the initial drop in arterial pressure, the IFD decreases significantly ($P<0.01$) in lumbricals fixed *in situ* (Figure 23, 24). Below 70 mm Hg, the IFD in both series I (Figure 23) and series II (Figure 24) returns to normal.

Adductor muscle appears to have a more stable response to hemorrhage. The interfilament distance does not change in adductor muscle fixed *in situ* (Figure 26). However, the IFD decreases significantly in adductor fixed by immersion. This decrease begins at 70 mm Hg ($P<0.05$) and is progressive. At 45 mm Hg the IFD has decreased to 46.6±0.4 nm (n=8) ($\bar{x}$±SEM) from 48.3±0.3 nm (n=8) at normal arterial pressure ($P<0.01$). In series VI which also represents adductor muscle fixed by immersion, the IFD has increased significantly at 30 mm Hg ($P<0.01$).

Therefore the interfilament distance in lumbricals has returned to normal at the lowest arterial pressure reached during early hemorrhagic shock. The same may be said for adductors if the increase in series VI is balanced against in the decrease in series V.

Sarcomere Length

Examination of Figures 23 to 28 reveals significant changes in sarcomere length. In Figures 23, 24, 26 and 28, there are significant ($P<0.01$) increases in Ls with the initial decrease in arterial pressure. There is also a significant increase in Ls when the arterial pressure is 45 mm Hg (Figure 27). It is only lumbricals fixed by immersion (Figure 25) which show an initial decrease ($P<0.01$).

With the progressive decrease in arterial pressure, the sarcomere length varies. Only in lumbricals fixed by arterial perfusion of 2.5% glutaraldehyde (Figure 24) is the final Ls normal. In series I (Figure 23), III (Figure 25), and IV (Figure 26) the final Ls is significantly decreased ($P<0.01$). In series V and VI (Figures 27 and 28), both involving adductors fixed *in vitro*, there is a significant increase in Ls ($P<0.01$).

Therefore, Ls is decreased in lumbrical muscle fixed *in situ* (Figure 23) or *in vitro* (Figure 25). In contrast, Ls in adductors is decreased for *in situ* fixed tissue (Figure 26) and increased in *in vitro* fixed tissue (Figure 27, 28). Consequently muscle fixed *in situ* have a reduced Ls as shock progresses. Ls for *in vitro* fixed muscle shows a variable response, perhaps due to the lack of control over muscle contraction during fixation.

Unit Cell Volume

The lattice structure of skeletal muscle has been shown to behave in an isovolumic manner (April *et al.*, 1971). An inverse relationship between sarcomere length and interfilament distance results in a constant unit cell volume. Therefore a reciprocal change in sarcomere length and interfilament distance would be expected as shock progressed.

Analysis of Figures 23 to 28 shows that the inverse relationship between sarcomere length does not hold as shock progresses. In Series I, a significant decrease in IFD at 80 mm Hg is associated with a significant increase in sarcomere length. Consequently the unit cell volume remains normal. At 60 mm Hg, the IFD is 47.4±0.9 nm (n=6), not significantly different from the normal IFD of 44.7±0.8 nm (n=6) ($P>0.05$). However, sarcomere length has decreased significantly ($P<0.05$), from 2.10±0.01 μm (n=30) to 2.06±0.01 μm (n=30). On the basis of a significant reduction in Ls, a significant increase in IFD would have been expected if the isovolumic relationship is to hold.

The failure of the isovolumic relationship between Ls and IFD to hold as shock progresses is evident in the remaining series. This is particularly evident in Figures 26 and 28. In Series IV (Figure 26) significant changes in Ls are accompanied by normal IFD. In Series VI, there is a significant increase in the final Ls ($P<0.01$) and IFD ($P<0.01$) at the end of early hemorrhagic shock (Figure 28).

The results suggest that as shock progresses, the normal inverse relationship between sarcomere length and interfilament distance does not hold. Each appears to react independently to the effects of hemorrhagic shock.

B. DISCUSSION

No significant change in the interfilament distance at the end of the early stage of hemorrhagic shock is suggested by the results. This would indicate no change in cell volume during this phase of shock. These results confirm previous studies showing no ultrastructural evidence of cell edema during early shock (DePalma *et al*, 1970; Holden *et al*, 1965). Campion *et al* (1969) did demonstrate a rapid drop in membrane potential in rat thigh muscle from a normal of -89 mv to -71 mv within 15 minutes after the onset of shock induced by an acute hemorrhage. As a result of this drop in membrane potential he postulated a 'leakage' of sodium and water into the cell with consequent cell edema. However, confirmatory increases in muscle sodium and water during the early stages of shock have not been forthcoming (Newton *et al*, 1968; Rothe, 1970; Rocchio *et al*, 1973). There have been occasional reports indicating increases in cell water after shock (Campion *et al*, 1969; Shires and Carrico, 1966) but all these studies were done during the post-transfusion or stage III of shock and do not apply to our results.

In only one series (Series VI) was any attempt at measurement of fiber volume made during the later stages of shock. The interfilament distance (48.0±0.3 nm, n=10) was only 1.3% greater than normal (47.4±0.1 nm, n=8) (P>0.10) one hour after shock had been established with the arterial pressure at 30 mm Hg implying no change in cell water. However, sarcomere length remained increased at 2.10±0.03 μm (n=30), from a normal 1.98±0.02 μm (n=30). No measurements were made in the post-transfusion stage of shock.

A greater degree of credibility may be given to this estimation of fiber volume in late shock since more recent membrane potential studies in the baboon have shown no changes in membrane potential and cell water several hours after hemorrhage (Holliday, 1975).

Myofilament Lattice

April *et al* (1971) have shown that the isovolumic property of the myofilament lattice is intrinsic to the lattice. The results in our study suggest that this isovolumic property of the lattice is disrupted during shock. Consequently, acute shock results in changes in the extracellular or intracellular environment which interferes with the forces maintaining the lattice spacing or which interferes with contraction.

Interstitial sodium (Koven *et al*, 1970) and potassium (Haljamäe, 1970) increase during shock. This increase, coupled with a loss of water but not protein from the interstitial fluid during shock might increase the extracellular tonicity and osmolarity sufficient to affect the transmembrane osmotic gradient. April *et al*

(1972) have shown that the osmolarity has a significant and inverse relationship to interfilament distance.

If the significant changes in IFD during early shock (Figure 23, 24, 27 and 28) are extrapolated to the data presented by April *et al.* (1972) regarding the relationship of osmolarity to IFD, we find that no change in extracellular or intracellular osmolarity should occur with the changes in IFD noted. No change in intracellular osmolarity would be expected since the lattice volume of fibers with intact membranes is not due to changes in the osmotic strength of the internal medium (April *et al.*, 1972; Mond, 1955; Boyle and Conway, 1941).

If this extrapolated interpretation of no change in extracellular osmolarity is correct, then it contradicts what might be expected from the data presented by Koven *et al.* (1970). However, the increased interstitial sodium noted by Koven *et al.* (1970) might be associated with adsorption onto collagen and hence would not significantly affect the osmolarity of the extracellular fluid. The increase in interstitial potassium (Haljamäe, 1970) amounts to only 5-6 mEq/liter and could not change the tonicity or osmolarity sufficiently to produce change in the myofilament lattice.

The unit cell volume reflects the total osmotic behavior of the muscle fiber (April *et al.*, 1971).

The osmotic dead space in skeletal muscle fibers varies from 35% in amphibian muscle to 40% in crayfish muscle. The osmotic dead space for vertebrate muscle has not been defined, but might be similar. This osmotic dead space may be related to the volumes of the various cell organelles, the fraction of bound water within a cell or the gel nature of the cytoplasm (Harris, 1961). Any of these factors could be altered significantly during shock to disrupt the isovolumic relationship.

A change in the osmotic dead space as a result of intracellular fluid shifts (Carlsen *et al.*, 1961) is very unlikely (April *et al.*, 1971). This is confirmed later in our study. A variation in the osmotic coefficients of cytoplasmic proteins (Dick, 1955) or a disruption of the gel nature of the cytoplasm would be expected to alter the intracellular osmolarity. As shown previously however, not only does a change in osmolarity appear not to occur, but changes in intracellular osmolarity probably do not affect the myofilament lattice significantly. Consequently, if a change in the osmotic dead space within muscle during shock occurs, it should not produce any significant disruption of the isovolumic relationship.

Intracellular and extracellular pH have a marked effect on the isovolumic relationship (April *et al.*, 1972). Couch *et al.* (1971) showed with pH microelectrodes inserted into the interstitial space, that the interstitial fluid pH decreased considerably during the early stage of shock. He also suggested that the intracellular pH would also decrease.

If the significant changes in IFD during shock (Figure 23, 24, 27, 28) are extrapolated to the data presented by April *et al.* (1972) regarding the relationship of IFD and pH, no significant change in extracellular pH would be evident during the early stage of shock. However, the intracellular pH might decrease rapidly to as low as 6.6 with the initial drop in capillary pressure. With further drops in arterial pressure, the capillary pressure is re-established with some improvement in perfusion. This would result in an increase in pH to normal due to a washout effect, and a normal IFD which is evident in most series.

The effects of a drastic change in pH during shock could be multiple. In muscle, including myocardium, the build up of hydrogen ions could interfere with the negative electrostatic charge between myofilaments. It is then postulated that negative electrostatic repulsive forces maintain the lattice spacing (Elliott et al., 1970). A build-up of positive hydrogen ions would reduce the negative charge and result in a decreased interfilament distance. Such a decrease in IFD is seen in Figures 23, 24 and 27. A decreased pH may inhibit calcium release by the sarcoplasmic reticulum; inhibit myosin-ATPase involved with contraction; interfere with numerous aerobic enzymes, converting the cell to anaerobic metabolism; and if the decreased pH is very severe, denature cytoplasmic and contractile protein. All would result in a disruption of normal excitation contraction coubling. It is obvious that a signficiant drop in intracellular pH can explain the disruption of the isovolumic property of the myofilament lattice. Changes in osmolarity or tonicity may occur during shock but there effect on the myofilament lattice would be minimal.

C. CONLUSIONS

1. The major conclusion from the results suggests that the normal

property of the myofilament lattice structure is disrupted during shock.

2. Intracellular water at the end of the early stage of shock returns to normal following significant decreases in cell water with the initial drop in arterial pressure.

CHAPTER IX

CELL MORPHOLOGY

A complete morphometric analysis of the distribution of cell organelles was performed on muscles from normal rats and rats subjected to hemorrhagic shock. By measuring organelle volume within different regions of the cell and determining sarcomere distribution, it was hoped that one might be able to discern local as well as general subcellular responses to hemorrhage and relate any changes to specific etiologic possibilities such as capillary perfusion abnormalities, acidosis or hypoxia. In addition, such analysis would allow correlation with known changes in extracellular and total water content of the muscle.

A. RESULTS

Mitochondria

The volume of mitochondria within each sarcomere was obtained by adding the volume within the A-band and within the I-band of each sarcomere. A Z-line was included within each micrograph of an I-band, thus yielding a full I-band section for analysis. The volume of mitochondria within a cell (ST) was obtained by first measuring the volume per sarcomere adjacent to the sarcolemma and near a capillary (SN) and the volume in the center of the cell (SA). It was anticipated that this analysis would yield information of any regional changes in the cell which might occur. ST was then obtained by averaging SN and SA:

$$ST = \frac{SN + SA}{2} \qquad (14)$$

Values for mitochondrial volume are expressed as fractions of total muscle volume.

Mitochondria account for 10.1% and 10.5% of tissue volume in both lumbrical and adductor muscles respectively fixed *in situ* (Tables 21 and 22). For muscle fixed *in vitro* by immersion in 2.5% glutaraldehyde, mitochondrial volume is 11.0% and 11.7% of muscle volume. These volumes do not differ significantly from the mitochondrial volumes of the respective muscle fixed *in situ*. The majority of mitochondria in both muscles are adjacent to the edge of the cell with lumbricals having a greater fraction of their mitochondria adjacent to the cell membrane. The peripheral to central ratio for mitochondria is 1.6:1 and 2.0:1 for adductors and lumbricals fixed *in situ* and 1.14:1 and 1.6:1 for lumbricals fixed by immersion.

The distribution of mitochondria within each sarcomere is only slightly different for the two muscles (Table 23). In both muscles mitochondria are predominantly in the I-band. Adductors and lumbricals contained 88%-93% and 85%-88% respectively of their mitochondria in the I-band when fixed by vascular perfusion. Whereas adductors fixed by immersion demonstrate little difference in the I-band predominance (83%-90%) from the same tissue fixed by arterial perfusion, lumbricals fixed by immersion are slightly different, with only 58%-94% of mitochondria within the I-band. This difference is related only to a significant difference in total A-band volume, with AT greater

($P<0.10$) in muscle fixed by immersion (Table 21).

Sarcoplasmic Reticulum

The volume of sarcoplasmic reticulum within the cell is similar irrespective of muscle type or method of fixation (Table 24 and 25). The mean volumes for adductors, 7.2% and 6.7% for arterial perfusion and immersion respectively are not significantly less than for lumbricals (7.2% and 7.8%) fixed by similar methods.

In contrast to the preponderance of mitochondria in the periphery of the cell and in the I-band, SR is much more evenly distributed within the cell. There is no difference in peripheral and central areas for adductors or lumbricals, fixed *in situ* or *in vitro*. The I-band contains 66% of sarcomere SR in adductors and 60% of sarcomere SR in lumbricals, a difference which is not significant. The significantly greater volume in the I-band ($P<0.05$) represents the presence of the terminal cisternae in the I-band.

B. DISCUSSION

The different methods of fixation have no demonstrable effect on SR volume or on the distribution of SR within the cell. Also SR is much more evenly distributed within the cell, reflecting its role in muscle contraction, in contrast to mitochondria which are very unevenly distributed.

Since adductors fixed by the two methods have the same volumes and distributions of mitochondria and the fixatives are the same, then some difference during fixation must account for the different volume of mitochondria in the A-band of lumbrical muscle fixed by immersion.

The only differences during fixation involved longer biopsy times for the latter muscles. Whereas less than 15 seconds was usually required to biopsy adductors and immerse in fixative, more than three minutes was frequently required to dissect the lumbricals from the carpal tunnel and then immerse in fixative. This dissection was also much more traumatic than required for adductors. Normal appearing mitochondria in the center of the cell, the same fiber volumes and the same volumes of SR (Tables 24, 25) in both tissues argues against a hypoxic effect during this period of biopsy.

Why such a local change in mitochondria? Several explanations must be considered. First, differences noted above are artifact due to insufficient sampling of animals. However, the difference in A-band volume noted above was for muscle from the same animal. The precision and objectivity of the stereologic methods used for analysis and the statistical significance suggest that a real difference does exist. Second, an increase in bound water may account for the increase in unit cell volume and for an increase in the A-band mitochondrial volume. However this should result in a general increase in mitochondrial volume unless mitochondria in the area adjacent to the cell membrane are directly involved with regulation of cell water in addition to their involvement in excitation-contraction coupling and energy production. In this situation local damage due directly to trauma during dissection or indirectly to hypoxia, both of which may result in cell membrane damage and shift of water into the cell, might result in an increased function and volume of mitochondria adjacent to the cell. A shift of mitochondria from the central to peripheral areas of the cell, particularly the A-band may reflect a recruitment to aid in this function. Both shifts appear to have occurred in lumbricals fixed by immersion and submitted to a stress during fixation not encountered by other tissues. The increase in mitochondria volume in the A-band adjacent to the sarcolemma corresponds to the area where not only close approximation of mitochondria and sarcolemma have been described (Sommer and Johnson, 1970; Walker et al., 1970; Bracker and Grove, 1971), but direct communication of mitochondria and extracellular space is suggested (Plate 19).

If mitochondria have a specific volume regulating function, then shifts in cell water may initially be controlled by mitochondrial recruitment and increased function and only after saturation of areas of cell binding and of mitochondrial function would an increase in fiber volume be evident.

The role of sarcoplasmic reticulum and mitochondria in excitation-contraction coupling is well defined (Peachey, 1965a, 1965b; Sandow, 1970; Close, 1972). However, the marked difference in distribution of the two organelles in the sarcomere and in the cell suggests a similar yet separate function. The paucity of mitochondria in the A-band suggests that calcium uptake after contraction is a minor role for mitochondria with a more major role for SR. Alternatively, since mitochondria and SR appear to communicate directly, the role of mitochondria may be facilitation of calcium uptake by the SR in the A-band rather than as a primary site of calcium uptake. The predominance of mitochondria in the I-band reflects their importance in providing high energy phosphates for calcium release from the terminal cisternae of the SR. In this respect, the direct communication between the two organelles shown previously would facilitate transport, not only of these high energy materials, but also transport of both substrates and products of metabolism from one to the other, thereby increasing the efficiency of the system.

The concept of SR being an extension of the extracellular space was presented previously. The close relationship of SR and mitochondria both in cellular distribution, function and structural continuity noted previously, would also imply continuity of the intermembranous compartment of the mitochondria with the extracellular space. Such a direct communication is occasionally visible (Plate 19).

The accessibility of the extracellular space to the endomembrane system has been probed with numerous electron dense materials, usually without success. Use of auto-radiography for localization of small molecule accessibility awaits better methods of identifying the position of the silver granules produced. However, in view of a possible direct communication of mitochondria and the extracellular space, entrance of large molecules such as horse-radish peroxidase or ruthenium red, both of which penetrate the SR and occasionally the mitochondria, may be by way of either the triad junction as suggested by Birks and Davey (1969) or by way of direct communications of the organelles with the sarcolemma.

C. CONCLUSIONS

1. The volume and distribution of sarcoplasmic reticulum is the same for lumbrical and adductor muscles.

2. The volume of mitochondria within lumbrical and adductor muscle is the same. However, lumbricals have a greater percentage of mitochondria in the periphery of the cell.

3. *In vitro* fixation may result in a different distribution of mitochondria with the sarcomere, compared to *in situ* fixation. However, *in situ* and *in vitro* fixation do not have a specific effect on the volume fraction of SR in either lumbrical or adductor muscle.

CHAPTER X

CELL MORPHOLOGY DURING SHOCK

Mitochondrial and sarcoplasmic reticulum volumes should respond to an acute hemorrhage in a fashion similar to the response of cell volume if the organelles are 'intracellular' and in osmotic equilibrium with the cytoplasm. If, for example, the IFD were to increase by 5%, then a 5% increase in mitochondria and SR volume might be expected if normal functional relationships are retained during shock. This is not the case.

The overall changes in cell volume (IFD), unit cell volume (Ks), sarcomere length (Ls) and mitochondrial and SR volumes are shown in Table 26 for all series. The difference in volume (ΔV) for each parameter between the normal control and the lowest arterial pressure obtained following hemorrhage is expressed both as a percentage of original organelle volume and of original cell volume. Changes in each parameter, with changes in the arterial pressure, will be discussed later.

The response of Ks, Ls and IFD to hemorrhage were discussed in a previous section. Reasons for the lack of correlation between the responses of each were also presented. In summary, although significant changes in IFD occurred as arterial pressure decreased, no significant changes in cell water would be evident at the end of the early phase of hemorrhagic shock, partially confirming previous reports both histological and analytical of no change in cell water at this early stage.

Examination of Table 26 yields the impression that no consistent relationship between cell volume (IFD) and SR volume exists during hemorrhage. SR volume changes are significant at the lowest arterial pressures in each series with the exception of Series II. Cell volume and SR volume have changed in opposite directions in Series I and III but in the same directions in Series V and VI. In Series IV SR volume has decreased significantly ($P<0.001$) whereas cell volume has not changed. Similarly SR volume and sarcomere length change significantly but in opposite directions in Series III and V but in the same direction in Series I, IV and VI. In Series II sarcomere length has increased significantly ($P<0.001$) whereas SR volume has not changed. Consequently, the significant positive relationship expected between SR volume and the lattice parameters is not evident.

Mitochondrial volume does not change significantly following hemorrhage with the exception of slightly significant decreases in Series I and III ($P<0.10$). VM and IFD change in opposite direction in Series I but in the same direction in Series III. Significant changes in IFD in Series V ($P<0.01$) and VI ($P<0.001$) are not associated with any significant changes in mitochondrial volume.

There are significant decreases in Ls in Series I ($P<0.05$) and Series III ($P<0.001$). Although this would suggest a significant positive relationship between mitochondrial volume and sarcomere length the significant changes in Ls in the remaining series without any change in mitochondrial volume, invalidates such an assumption.

The close structural relationships between mitochondria and SR noted previously are not re-inforced when the changes in volume of the two organelles are examined (Table 26). In only Series I is a significant and positive relationship suggested. Opposite changes in volume occur in Series III. The remaining series (except Series II) illustrate significant changes in SR volume but not mitochondrial volume.

The apparent autonomy of cell organelle volume changes, from cell volume change during shock described above, is strongly re-inforced when the changes in VM and VSR (Figures 29 to 40) are compared to their respective lattice parameters (Figures 23 to 28) in each series as the arterial pressure decreases.

No association between VM and the lattice parameters is evident for Series I, II and VI. However, some association of VM and the lattice parameters might be present in Series III, IV and V. Some similarity between VSR and the lattice parameters may be implied for Series V and VI but not Series I to IV.

Confirmation or denial of these subjective impressions and those obtained from Table 26 was attempted by cross-correlation of VM or VSR with each lattice parameter. The cross-correlations were performed on an IBM 470 computer.* Correlations of VM or VSR with IFD or VI were accomplished using individual paired values from the same sections. However, since Ls is measured from different sections, only mean values of Ls were used to correlate with the mean of either VM or VSR. Table 27 illustrates the correlation coefficients and the significance of each.

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*The author gratefully acknowledges the assistance of Dr. M. Grace and Mr. J. Hansen of the W.W. Cross Cancer Institute in performing these statistical correlations.

In none of the series is there any significant correlation between total mitochondrial volume (VM) and IFD. However, in Series VI, a significant correlation between VM in the A-band and IFD is present ($r=0.79$, $P<0.05$). Mitochondrial volume and sarcomere length are also unrelated with the exception of Series IV where VM and Ls show a strong positive correlation ($r=0.86$, $P<0.01$). However, this significant relationship is due only to a strong correlation of VM in the I-band and sarcomere length ($r=0.89$, $P<0.01$) since there is no correlation of A-band mitochondrial volume and Ls ($r=0.07$). In Series II, a significant negative correlation is evident for VM in the A-band and Ls ($r=-0.80$, $P<0.10$).

If total SR volume is compared to either IFD or Ls as shock progresses in each series, no significant positive or negative relationships between SR and the lattice parameters are evident. This is also true for A-band VSR and IFD or Ls. However, I-band VSR has a significant negative relationship with IFD in Series III ($r=-0.84$, $P<0.05$) and IV ($r=-0.63$, $P<0.10$) and with Ls in Series III ($r=-0.82$, $P<0.05$) and V ($r=-0.73$, $P<0.10$).

There is no consistent correlation between VSR and VM. In Series II there is a significant negative relationship ($r=-0.94$, $P<0.01$) between the two organelles but all other series exhibit neither a positive nor a negative relationship. Examination of each area of the cell results in similar findings. A-band VSR and VM show a positive correlation in Series V (r=0.73, P<0.05) and Series III (r=0.91, P<0.01). However, the latter is confined to the center of the cell only. In contrast to this positive A-band correlation, I-band VSR and VM show a negative relationship in Series II (r=-0.97, P<0.001) and Series III (r=-0.69, P<0.10). In each however, this negative relationship is confined to the subsarcolemmal area only (r=-0.96, P<0.001 and r=-0.87, P<0.01 in Series II and III respectively). Most of the remaining series have negative, but not significant, correlation coefficients for I-band VSR and VM.

A. DISCUSSION

During a normal contraction-relaxation cycle, calcium is initially transported from SR cisternae into the sarcoplasm following membrane depolarization. A shift of water with the calcium occurs and should result in a decrease in I-band SR volume and a slight increase in IFD or cell volume. During relaxation there is an uptake of calcium and water by the SR and perhaps A-band mitochondria. Consequently, one would expect an inverse relationship between IFD and VSR and a direct relationship between VSR and Ls.

The inverse relationship in Series III and IV between IFD and I-band VSR is therefore expected. However, in none of the other series is such a relationship evident. Furthermore, a significant inverse relationship between VSR and Ls is seen in Series III (r=-0.82,

$P<0.05$) and V ($r=-0.73$, $P<0.05$) which is opposite to the expected positive relationship.

Several conclusions may be obtained from the above. The normal relationship between SR volume and contraction may be disrupted during early shock. However, inhibition of muscle contraction has not been demonstrated until the preterminal phases of shock. Consequently if a disruption of normal contraction is present, it is either reversible or of minimal significance in the early stages of shock.

Alternatively, the results support the concept that SR is not an 'intracellular' organelle. Changes in SR volume during early shock may also reflect impaired diffusion (Koven *et al*, 1970). Significant changes in cation content within the SR, such as variation in calcium concentrations, could result in loss of expected SR-cell contraction relationships. Estimation of the ion content of SR during the different phases of shock is required to confirm or deny this possibility.

There is little evidence in this study to confirm that mitochondria are 'intracellular'. It is known that mitochondria are involved in calcium uptake during relaxation of skeletal muscle. Consequently, VM and Ls should show a positive correlation and VM and IFD a negative correlation. In only Series IV was the expected positive correlation for VM and Ls seen. In Series II a negative correlation between A-band VM and Ls was found ($r=-0.80$, $P<0.05$). In Series VI, a positive correlation between A-band VM and IFD was found. Consequently, one cannot assume that changes in VM are a reflection of changes in cell volume.

The positive correlation between VM and VSR in the A-band in Series III and V is expected. Both organelles are involved in calcium uptake during relaxation. The very significant inverse relationship between VM and VSR in the I-band (Series II, III) may be explained by assuming that immediately prior to or during excitation of the SR cisternae, there is a shift of calcium and water from mitochondria to SR due either to a high concentration gradient or a sudden mass transfer of fluid. The latter may be due to either a high electrochemical gradient between the two organelles or it may involve a mass propulsion of fluid which would require some form of contractile protein associated with the endomembrane system.

The failure for VM and VSR to correlate in either a positive or negative way in all series may be due either to insufficient sampling, or alternatively during shock, the normal structural and/or functional relationships between the two organelles is disrupted. Baue *et al* (1972) have shown a 2.5 fold increase in mitochondrial sodium. Unfortunately they did not measure SR sodium or cell sodium. If a structural or functional disruption of the normal mitochondrial-SR relationship occurred during shock, one might expect a disparity in ion content between the two organelles. The high mitochondrial sodium would inhibit oxidative phosphorylation (Baue *et al*, 1972). If the 'perfusion' of mitochondria were dependent on normal extracellular fluid pressure and flow gradients along the cell, then increased mitochondrial sodium could be a result of decreased 'perfusion' of the endomembrane system during shock. It has been shown that hypoxia per se will not result in either biochemical or structural alterations seen in shock (Schildt, 1972).

Reduced perfusion is a prerequisite for developing such changes.

Therefore, if one assumes that the endomembrane system is extracellular, reduced 'perfusion' of the endomembrane system during shock might result in significant changes in ion content within the endomembrane system which would inhibit metabolism before significant hypoxia would be present. Although this is only theoretical, the above assumptions regarding the endomembrane system are compatible with the metabolic changes which are known to occur in shock.

If the endomembrane system were extracellular as suggested above, then some evidence should be present to confirm this. Cross-correlation of VSR and VI during shock shows a positive relationship only in Series VI ($r=0.68$, $p<0.10$). However, VM and VI have a significantly positive relationship in Series III ($r=0.84$, $p<0.05$) and IV ($r=0.91$, $p<0.01$). This is due to the relationship of VI and VM along the sarcolemma but not in the center of the cell ($r=0.92$, $p<0.01$ and $r=0.94$, $p<0.001$ in Series III and IV respectively). Consequently, the endomembrane system, particularly mitochondria, may communicate directly with the interstitium as suggested previously. Therefore, the endomembrane system may be influenced by those factors affecting the 'extracellular' space rather than the 'intracellular' space.

3. CONCLUSION

The relationships between the sarcoplasm, the endomembrane system and the interstitium is complex and much more so in shock. There is some evidence to suggest that the endomembrane system functions as an extension of the extracellular fluid. The normal organelle, cytoplasmic relationships may also be disrupted during shock. Whichever, one must not assume that changes in organelle volume reflect changes in cell volume.

CHAPTER XI

EXTRACELLULAR SPACE

Normal Muscle

The interstitial space volumes in lumbrical and adductor muscles fixed with 2.5% glutaraldehyde at normal arterial pressures are 18.2±3.8% (n=25) and 7.8±1.4% (n=22) respectively (Table 15). These values include tissue fixed by arterial and immersion methods of fixation since there is no significant difference in VI between the two methods.

The volume of extracellular space measured by chemical means varies inversely with the size of the marker molecule being used (Cizek, 1968). The histological extracellular fluid volume does not include vascular volume. In comparison to *in vitro* measurements of extracellular fluid volume in muscle, the volumes obtained for lumbrical and adductor muscle are equivalent to measurements made with medium to large molecular weight substances. Lumbrical extracellular space volume is equal to the inulin space. Adductor extracellular space volume is equal to albumin space. Both are considerably less than the extracellular space measured with small molecular weight markers such as chloride and sulfate even if a shrinkage factor of 10% (Davey, 1973; April *et al.*, 1972) is included for fixation artifact.

If the contention by Birks and Davey (1969) is correct; ie. that sarcoplasmic reticulum is connected to the extracellular space, then VSR plus VI should equal the extracellular space volume obtained by using small molecular weight markers. The extracellular fluid volume obtained by this summation is 24.0% and 16.3% for lumbrical and adductor muscle fixed *in situ* and 27.4% and 12.8% for the same muscles fixed *in vitro*. Allowing for a fixation shrinkage factor of 10%, only the volumes for lumbricals are sufficient to explain the small molecular weight extracellular space.

In previous chapters, the continuity of mitochondria with sarcoplasmic reticulum and the extracellular space was described. If mitochondrial volume is included in the estimation of extracellular fluid volume, this 'total' extracellular space volume (VI+VSR+VM) is 25.0% and 26.8% for lumbrical and adductor muscle respectively fixed *in situ* and 37.5% and 24.5% for the same muscle fixed *in vitro*. These volumes are within the range reported for extracellular space volume obtained using small molecules such as sulfate and chloride.

The final phase of radio-sulfate distribution represents the "non-functional" phase of extracellular fluid and accounts for 15% of extracellular fluid volume (Middleton *et al.*, 1969). Sarcoplasmic reticulum volume accounts for 7.6% of adductor muscle volume and 8.4% of lumbrical muscle volume, as seen in Chapter 10. Since muscle accounts for 42% of total body water (Cizek, 1968), SR then accounts for 9.6% (adductor) to 10.6% (lumbrical) of total extracellular fluid volume, assuming that extracellular fluid accounts for 30% of total body water (Cizek, 1968). This is less than the 15% described for the non-functional phase of extracellular fluid.

If the volume of mitochondria were to be included as part of the extracellular space, then mitochondria would account for 13% (adductor) and 14.4% (lumbrical) of extracellular fluid volume. However, a considerable fraction of mitochondrial volume is taken up by membranes and matrix. Assuming that the volume of the intermembranous space and cristae are only 30%, they could contribute 4.2% (adductor) to 4.3% (lumbricals) of extracellular fluid volume. Consequently, VSR+VMc, where VMc is 30% of total mitochondria volume (VM), could account for 13.8% (adductor) to 14.8% (lumbrical) of extracellular fluid volume. This is almost identical to the 15% estimated for the 'non-functional' component of extracellular fluid.

It is possible therefore that the endomembrane system of the cell, particularly the SR and mitochondrial components, is the site for the 'non-functional' phase of extracellular fluid.

Extracellular Space Volume During Shock

Interstitial volume (VI) in each series is shown in Figures 41 to 46 as shock progresses. There is a significant decrease in VI for all series with the exception of Series VI (Figure 46) whose VI is apparently normal. However, since VI decreased in other cell series, the apparently normal value at 30 mm Hg probably reflects a significant increase from what should be a significant decrease. This increase probably reflects the trauma of repeated biopsy and would be analogous to the VI in a contused muscle. Consequently, it is questionable whether serial biopsies from the same muscle should be used for measuring extracellular fluid, either histologically or by chemical means.

The decrease in interstitial volume in Series I to V (Figures 41 to 45) follows very closely or is coincident with sudden decreases in capillary pressure. This is expected and confirms the rapidity with which Starling's hypothesis functions. The final interstitial space volume averages 70% in Series I to III (Figures 41 to 43; lumbrical muscle) and 56% in Series IV and V (Figures 44, 45; adductor muscle). The average decrease for *in situ* fixed muscle is 65%. Expressed in terms of muscle volume, this decrease amounts to an average 11% of muscle volume. Assuming that muscle contains 42% of total body water (Cizek, 1968), then the average decrease in VI for *in situ* fixed muscle is 4.6% of total body water. With extracellular fluid volume equal to 30% of total body water, the average decrease in VI for *in situ* fixed muscle is 15.4% of total extracellular fluid volume.

The decrease in total extracellular fluid volume of 15.4% is 30.5% greater than the volume decrease expected on the basis of blood loss alone which was 11.8% of extracellular fluid volume (Cizek, 1968). This agrees with Shires *et al.* (1960) who reported a 43% greater decrease in extracellular fluid volume than could be explained by blood loss alone.

If the endomembrane system is functionally extracellular as the results in preceding chapters would suggest, then analysis of the 'total' extracellular fluid volume change might yield some information which could explain the discrepancies between the work by Shires and other authors.

'Total' extracellular fluid volume (VT) (VI+VSR+VM) decrease was estimated by including only the significant changes in each component. There was a significant decrease in VT in all of Series I to V. Series VI was excluded because of the possibility of inaccurate VI due to the repeated trauma of biopsy. VT decreased in lumbricals by 31%, 26% and 25% in Series 1 to III respectively. For adductors in Series IV and V, the decreases were only 12% and 8% respectively.

The decreases in VI for adductor muscle is the same as the estimated decrease due to blood loss alone. On the other hand, the decrease in VT for lumbricals is 2.1 to 3.8 times greater than estimated decrease in blood volume.

The diffusion of water soluble substances through the interstitium is reduced as shock progresses (Koven *et al.*, 1970; Appelgren, 1972). Consequently, tracer equilibration times will vary during shock. It appears therefore that measurement of extracellular fluid during shock is governed not only by equilibration times for tracers but also by the type of muscle used for analysis, the severity of shock and the stage of shock.

The results of this project would suggest that the 'non-functional' phase of extracellular fluid resides in the endomembrane system of the cell. However, a correlative study involving tracer and histological analysis of 'VT' in the same muscle must be carried out to confirm this impression in both normal and shock states. Since we have made no measurements during the late or post-transfusion stages of shock, we cannot confirm or deny reported findings for extracellular or cellular fluid changes in late shock.

CHAPTER XII

SUMMARY AND DISCUSSION

The successful treatment of patients in shock requires an accurate knowledge of the fluid and electrolyte shifts which occur at the capillary and cell levels during shock. However, these fluid shifts have not been defined with accuracy. Consequently, there is considerable controversy regarding the rate, type and volume of fluids which should be used in the treatment of shock.

The failure to define the fluid shifts during shock stems from an inability to accurately measure extracellular fluid volume, not only during shock but also in the normal state. Numerous materials and methods have been used in an attempt to measure and define the components of the extracellular fluid space. Plasma volume has been accurately measured. However, interstitial fluid volume and a 'non-functional' component of extracellular fluid have not been well defined due to an inability to measure or define the location of this 'non-functional' component. Since measurement of intracellular fluid is dependent on accurate measurements of extracellular fluid, the fluid spaces measured during shock are, at best, only approximations. The only consistent finding from all studies has been the inverse relationship between the extracellular fluid volume measured and the size of the substance used to measure the extracellular fluid volume.

Several authors have suggested that the difficulties associated with the measurement of extracellular fluid could be resolved if a portion of the intracellular fluid was accessible to small molecules but not large molecules. Birks and Davey (1969) demonstrated that sarcoplasmic reticulum (SR) in muscle cells may be extracellular. They suggested that the SR may be the site for the 'intracellular' fluid available to small molecules.

SR volume in mammalian muscle is approximately equal to the volume of the 'non-functional' component of extracellular fluid. SR has been shown to swell during shock. Consequently, changes in SR volume during shock, coupled with the possibility that SR is extracellular, may explain many of the conflicting reports of extracellular fluid volume change during shock.

Examination of the ultrastructure of skeletal muscle revealed evidence of direct continuity of sarcoplasmic reticulum and the intermembrane space of mitochondria. There was also a suggestion that mitochondria may communicate directly with the interstitial space. Consequently, the endomembrane system of the cell may be an extension of the extracellular space.

A stereologic analysis of cell volume, organelle volume and extracellular fluid volume confirmed the ultrastructural evidence that the endomembrane system functions as an extension of the extracellular fluid compartment. Whereas cell volume remained essentially constant during the early stages of acute hemorrhagic shock, mitochondrial volume and SR volume changed significantly. Furthermore, a highly positive correlation of the mitochondrial volume and interstitial volume was found. Therefore, changes in mitochondrial and SR volume during shock are not synonymous with changes in cell volume.

The results also indicate that the volume of the endomembrane system is equivalent to the volume of the 'non-functional' component of extracellular fluid. The histological measurement of extracellular fluid equates with reported chemical estimations only if the endomembrane system is included as part of the extracellular fluid. Therfore, the inverse relationship between molecular size and measured extracellular fluid volume is explained by assuming that small molecules may penetrate the endomembrane system, but large molecules do not.

The conflicting reports of changes in extracellular fluid during shock can also be explained if the endomembrane system is extracellular. Since diffusion is impaired during shock, short equilibration times would not allow penetration of an extracellular marker into the endomembrane system (EMS). Consequently the extracellular fluid volume measured would be less than the volume which actually existed. One might conclude, therefore, that there was a greater loss in extracellular fluid volume than could be accounted for by blood loss alone. Long equilibration times would result in a measurement of 'total' extracellular fluid volume (interstitial space and EMS volume). In this situation, no change in extracellular fluid volume would be noted other than the decrease due to blood loss alone. It is apparent therefore that one does not need to involve an increase in intracellular fluid (Campion *et al*, 1969) to explain extracellular fluid changes during shock if it is assumed that the EMS is extracellular.

The probability that the EMS is 'extracellular' is fully compatible with known changes in organelle structure during shock. Swelling of these organelles during the late stages of shock may be due to an increase in sodium and water content similar to what occurs in the interstitium. The importance of a high sodium content within the EMS (Baue *et al*, 1972) lies in the possibility that high sodium levels may inhibit various mitochondrial enzymes, particularly those associated with aerobic metabolism. If this is so, then are the metabolic changes seen in shock due to hypoxia, as currently believed, or are they a result of cation inhibition secondary to reduced 'perfusion' of the EMS during shock?

The preceding discussion illustrates that the possible extracellular nature of the EMS is entirely compatible with present knowledge about shock. This concept not only resolves the controversies surrounding fluid shifts during shock, but it also provokes a re-evaluation of our current thinking regarding the 'cell' changes during shock.

TABLES

TABLE 1

TYPES OF SHOCK

aerial
allergic
amniotic fluid
anaphylactic
anaphylactoid
anaesthetic
apoplectic
asthmatic
barium
bomb
break
burn
cardiogenic
cerebral
chronic
cold
colloid
colloidoclastic
compensated
cyclo
deferred
dehydration
delayed
diastolic
drum
electric
embolic
emotional
endotoxin
epigastric
epinephrine
erethismic
faradic
fetal
gravitation
gravity
heart
hematogenic
hemoclastic
hemolytic
hemorrhagic
histamine
hypertensive
hypervolemic
hypnoclastic
hypoglycemic
hypotensive
injection
insulin
intestinal
irreversible
liver
medical
mental
mesenteric artery
metabolodispersion
micro
neurogenic
normovolemic
obstetric
oligemic
osmotic
paralytic
peptone
peripheral vasomotor collapse
phenolic
pleural
postoperative
postpartum
primary
protein
psychic
railway
reversible
secondary
sense
septic
serum
sexual
shell
speed
spinal
static
surgical
terminal
testicular
thrombin
thyroxin
torpid
tourniquet
toxic
transfusion
traumatic
tumbling
uncompensated
warm
wound
vagovagal
vasoconstrictor
vasodilator
vasogenic

TABLE 2

CLASSIFICATION OF SHOCK

I. HYPOVOLEMIC:

   A. Pure

      1. Blood loss
      2. Plasma loss
      3. Water loss

   B. Complicated

II. CARDIOGENIC:

   A. Pure

      1. Failure of left ventricular ejection
         (a) infarction
         (b) arrhythmia
      2. Failure of left ventricular filling
         (a) tamponade
         (b) pulmonary embolism

   B. Complicated

      1. Electrolyte abnormalities
      2. Late hypovolemia
      3. Sepsis

III. PERIPHERAL POOLING:

   A. Epidural and general anaesthesia

   B. Loss of tone in resistance vessels

   C. Trapping in capacitance vessels (Endotoxin shock)

IV. CELLULAR DEFECT:

Decreased oxygen utilization despite high flow and normal oxygen content of blood (Septic shock).

TABLE 3

EXTRACELLULAR FLUID SHIFTS DURING SHOCK

NO CHANGE		INCREASE		DECREASE	
Anderson *et al*	1967,1969	Doty *et al*	1970	Albert *et al*	1967
Collins *et al*	1971	Newton *et al*	1969*	Carrico *et al*	1963,1966
Grable *et al*	1962	Pluth *et al*	1967	Crenshaw *et al*	1962
Gutelius *et al*	1968	Rothe	1970	Dillon *et al*	1966
Newton *et al*	1969*	Shizgal and Gutelius	1972	Mathews and Douglas	1963
Reid *et al*	1967			Shires *et al*	1960,1964
Roth *et al*	1967			Shires	1965
Schloerb *et al*	1967				
Serkes and Lange	1966				
Shizgal and Gutelius	1967				
Vineyard and Osborne	1967				
Virtue *et al*	1966				

* Increase in liver, muscle and connective tissue extracellular fluid but no change in total body extracellular fluid.

TABLE 4

INTRACELLULAR WATER DURING SHOCK

NO CHANGE		INCREASE		DECREASE	
Anderson *et al*	1967	Albert *et al*	1967	Johnson and Tucker	1968
Reid *et al*	1967	Campion *et al*	1969	Stewart and Rourke	1936
Rocchio *et al*	1973	Crenshaw *et al*	1962	Shizgal *et al*	1968
Slonim and Stahl	1968	Shires and Carrico	1966		
		Stahl	1967		
		Holden *et al*	1965*		
		DePalma *et al*	1970*		
		Hift and Strawitz	1961*		

*Ultrastructural studies demonstrating cell edema.

TABLE 5

NUMBER OF SAMPLES AND TIME REQUIRED FOR EACH EXPERIMENT

STANDARD SAMPLE PER	SPECIMEN	SECTION	MICROGRAPH	POINTS COUNTED	
				(P)	(g^2)
Electron micrograph				300	2,700
Section			9	2,700	24,300
Specimen		4	36	10,800	97,200
Experimental group	6	24	216	64,800	583,200
TIME REQUIRED PER					
Electron micrograph (minutes)	10				
Section (minutes)	90				
Specimen (hours)	6.0				
Experiment (hours)	36.0				

TABLE 6

NUMBER OF CALCULATIONS PER SAMPLE FOR EACH VARIANCE

Parameter	Accuracy (±) % (±2 S.D.)	Number of Measurements Required
VI	20	4
VC	20	8
VL	20	8
VN	20	6
VM	10	4
VSR	10	3
IFD	5	1

TABLE 7

LUMBRICAL MUSCLES FIXED WITH 1% GLUTARALDEHYDE*

Method of Fixation	VI (%)	VFD (nm)	VC (%)	VL (%)	VM (%)	VSR (%)
Arterial Perfusion	19.3± 2.12 (n = 4)	44.7± 0.8 (n = 6) (P < 0.01)	1.16± 0.23 (n = 4) (P < 0.10)	0.75± 0.07 (n = 4)	8.47± 1.32 (n = 6) (P < 0.05)	10.66± 1.49 (n = 6) (P < 0.01)
Carpal Tunnel	24.18± 7.06 (n = 4)	38.7± 0.4 (n = 10)	1.76± 0.22 (n = 4)	0.68± 0.22 (n = 4)	15.45± 1.61 (n = 9)	7.00± 0.76 (n = 9)

*Values expressed as % of tissue volume ($\bar{x} \pm$ S.E.M.)

n = number of measurements

TABLE 8

CAPILLARY AND INTERSTITIAL FLUID VOLUMES IN LUMBICALS AND ADDUCTOR MUSCLES

2.5% GLUTARALDEHYDE FIXATION

Method of Fixation	VI		VC	
	Lumbrical (%)	Adductor (%)	Lumbrical (%)	Adductor (%)
Arterial Perfusion	27.46 ± 2.20 (n = 4) ($P < 0.05$)	8.51 ± 1.96 (n = 5)	1.23 ± 0.21 (0.61 ± 0.09)* (n = 4)	1.34 ± 0.36 (0.69 ± 0.24)* (n = 5)
Immersion	18.87 ± 0.57 (n = 3)	8.69 ± 1.96 (n = 5)	1.17 ± 0.31 (0.54 ± 0.17)* (n = 3)	1.43 ± 0.10 (0.69 ± 0.07)* (n = 5)

Values expressed as % of tissue volume ($\bar{x}$ ± S.E.M.)

( )* = Capillary lumen volume

TABLE 9

EFFECT OF PERFUSION PRESSURE ON LUMBRICAL MUSCLES

Type of Perfusion	Perfusion Pressure (mm Hg)	VI (%)	VC (%)	VL (%)	VIFD (nm)
Pump	145	4.91 ± 1.07 (n = 4)	0.69 ± 0.13 (n = 4)	0.31 ± 0.04 (n = 4)	48.3 ± 0.05 (n = 11) (P <0.001)
Gravity	100	12.11 ± 3.40 (n = 7)	1.27 ± 0.32 (n = 7)	0.71 ± 0.22 (n = 7)	46.8 ± 0.04 (n = 6)
Pump	145	14.91 ± 1.93 (n = 6)	1.38 ± 0.26 (n = 6)	0.57 ± 0.14 (n = 6)	46.9 ± 0.05 (n = 6) (P <0.01)
Gravity	70	10.53 ± 2.45 (n = 4)	1.40 ± 0.02 (n = 4)	0.65 ± 0.01 (n = 4)	46.5 ± 0.07 (n = 9)

% = % tissue volume ($\bar{x}$ ± S.E.M.)

n = number of observations per muscle

TABLE 10

EFFECT OF PERFUSION PRESSURE ON CELL ORGANELLES. LUMBRICAL MUSCLE

Method of Perfusion	Perfusion Pressure (mm Hg)	VM			VSR			N
		AT	IT	ST	AT	IT	ST	
Pump	145	1.22 (± 0.39)	8.56 (± 1.43)	9.78 (± 1.69)	3.04 (± 0.16)	4.41 (± 0.17)	7.36 (± 0.25)	11
Gravity	100	1.21 (± 0.63)	8.18 (± 0.90)	9.39 (± 1.51)	3.23 (± 0.17)	4.43 (± 0.24)	7.76 (± 0.35)	6
Pump	145	1.18 (± 0.17)	8.65 (± 1.48)	9.69 (± 1.52)	2.44 (± 0.25) *	3.57 (± 0.11) *	6.01 (± 0.31) *	6
Gravity	70	2.03 (± 0.56)	7.10 (± 0.85)	9.14 (± 1.23)	2.95 (± 0.13)	4.31 (± 0.27)	7.15 (± 0.36)	9

Values expressed as tissue volume ($\bar{x}$±SEM)

* $P<0.10$

TABLE 11

EFFECT OF PERFUSION PRESSURE ON ADDUCTOR MUSCLE

Type of Perfusion	Perfusion Pressure (mm Hg)	VI (%)	VC (%)	VL (%)	VIFD (nm)
Pump	145	6.32 ± 2.53 (n = 4)	1.01 ± 0.22 (n = 4)	0.61 ± 0.10 (n = 4)	48.4 ± 0.04 (n = 12) *
Gravity	100	12.10 ± 3.73 (n = 4)	1.23 ± 0.28 (n = 4)	0.78 ± 0.22 (n = 4)	47.5 ± 0.05 (n = 9)
Pump	145	7.46 ± 1.34 (n = 4)	0.61 ± 0.22 (n = 4)	0.33 ± 0.10 (n = 4)	46.3 ± 0.05 (n = 8) *
Gravity	70	10.94 ± 1.73 (n = 7)	0.82 ± 0.14 (n = 7)	0.47 ± 0.08 (n = 7)	47.2 ± 0.06 (n = 9)

% = % tissue volume ($\bar{x} \pm$ S.E.M.)

n = number of measurements per muscle

* $P < 0.001$

TABLE 12

EFFECT OF PERFUSION PRESSURE ON CELL ORGANELLES. ADDUCTOR MUSCLE

Method of Perfusion	Perfusion Pressure (mm Hg)	VM			VSR			
		AT	IT	ST	AT	IT	ST	N
Pump	145	1.74 (± 0.95)	11.32 (± 1.27)	13.05 (± 1.84)	2.75 (± 0.14) +	4.35 (± 0.29)	7.10 (± 0.39) **	12
Gravity	100	1.02 (± 0.24)	14.35 (± 1.33)	15.59 (± 1.29)	1.84 (± 0.16)	3.91 (± 0.16)	5.70 (± 0.24)	9
Pump	145	0.55 (± 0.20)	4.10 (± 0.85)	4.65 (± 0.96)	2.84 (± 0.13) *	4.03 (± 0.28)	6.87 (± 0.33)	8
Gravity	70	1.04 (± 0.24)	5.03 (± 1.13)	6.07 (± 1.24)	2.43 (± 0.16)	4.67 (± 0.23)	7.09 (± 0.28)	9

Values expressed as % tissue volume ($\bar{x}$±SEM)

n = number of measurements for each organelle

+ $P < 0.01$

** $P < 0.05$

* $P < 0.10$

TABLE 13

INTERSTITIAL SPACE VOLUME VERSUS TONICITY

Buffer Tonicity	Adductor VI (% tissue volume)	Lumbricals VI (% tissue volume)
0.67 N	5.17 ± 1.90 (n = 4)	19.31 ± 2.12 (n = 4)
1.0 N	8.51 ± 1.96 (n = 5)	27.46 ± 2.20 (m = 4)

Values expressed as $\bar{x}$ ± S.E.M.

TABLE 14

EFFECT OF BUFFER TONICITY IN INTERSTITIAL SPACE VOLUME (VI)

Buffer Tonicity	VI (% tissue volume)
0.5 N	4.81 ± 0.75 (n = 5)
1.0 N	7.62 ± 0.84 (n = 5)
2.0 N	10.27 ± 1.10 (n = 5)

TABLE 15

NORMAL INTERSTITIAL FLUID VOLUME IN LUMBRICAL AND ADDUCTOR MUSCLE

Fixative	Method of Fixation	Lumbrical Muscle (% Muscle Volume)	Adductor Muscle (% Muscle Volume)
1% Glutaraldehyde	Arterial	19.31 ± 2.12 (n = 4)	5.17 ± 1.90 (n = 4)
	Carpal tunnel	24.13 ± 7.06 (n = 4)	
2.5% Glutaraldehyde	Arterial	27.46 ± 2.20 (n = 4)	8.51 ± 1.96 (n = 5)
		10.91 ± 2.07 (n = 4)	6.32 ± 2.53 (n = 4)
		12.11 ± 3.40 (n = 7)	12.10 ± 3.73 (n = 4)
		$\bar{x}$ = 16.83 ± 6.65 (n = 15)	$\bar{x}$ = 8.98 ± 1.68 (n = 13)
	Immersion	18.87 ± 0.57 (n = 4)	8.69 ± 1.96 (n = 5)
		20.31 ± 3.55 (n = 6)	3.50 ± 0.14 (n = 4)
		$\bar{x}$ = 19.59 ± 0.72 (n = 10)	$\bar{x}$ = 6.10 ± 2.59 (n = 9)
	Total	$\bar{x}$ = 18.21 ± 3.83 (n = 25)	$\bar{x}$ = 7.82 ± 1.42 (n = 22)

TABLE 16

CELL VOLUME (IFD) IN LUMBRICAL AND ADDUCTOR MUSCLE

Fixative	Method of Fixation		Lumbrical Muscle (nm)		Adductor Muscle (nm)
1% Glutaraldehyde	Arterial		44.7 ± 0.8 (n = 6)		42.5 ± 0.6 (n = 6)
	Carpal tunnel		38.7 ± 0.4 (n = 10)		
2.5% Glutaraldehyde	Arterial		47.1 ± 1.0 (n = 8)		43.3 ± 0.7 (n = 8)
			46.8 ± 0.4 (n = 6)		48.4 ± 0.4 (n = 12)
			48.2 ± 0.5 (n = 11)		47.5 ± 0.5 (n = 9)
		$\bar{x}$ =	47.4 ± 0.5 (n = 25)	$\bar{x}$ =	46.4 ± 1.6 (n = 29
	Immersion		47.7 ± 0.3 (n = 6)		48.3 ± 0.3 (n = 8)
			47.3 ± 0.2 (n = 8)		47.4 ± 0.1 (n = 8)
		$\bar{x}$	47.5 ± 0.2 (n = 14)	$\bar{x}$ =	47.8 ± 0.5 (n = 16)
	Total	$\bar{x}$	47.4 ± 0.3 (n = 39)	$\bar{x}$ =	47.0 ± 0.9 (n = 45)

TABLE 17

CELL VOLUME FRACTIONS AND INTERSTITIAL SPACE VOLUME IN HIND LIMB MUSCLES

Muscle	IFD (nm)	VM (%)*	VSR (%)*
Lumbrical	44.7 ± 0.8	8.47 ± 1.32	10.66 ± 0.61
Soleus	37.6 ± 0.3	17.13 ± 3.15	8.35 ± 1.19
E D L+	36.4 ± 0.5	10.92 ± 1.64	7.26 ± 0.59
Gastrocnemius	41.8 ± 0.6	12.36 ± 4.17	7.29 ± 0.77
Adductor brevis	42.5 ± 0.6	13.12 ± 1.96	7.26 ± 0.44

*% of muscle volume ($\bar{x}$ ± S.E.M.)

+ Extensor digitorum longus

n = 6 for each mean value

TABLE 18

NORMAL CAPILLARY VOLUME. LUMBRICAL AND ADDUCTOR MUSCLE.

Lumbrical Muscles				Adductor Muscles			
1% Glutaraldehyde		2.5% Glutaraldehyde		2.5% Glutaraldehyde			1% Glutaraldehyde
Arterial Perfusion	Carpal Tunnel Perfusion	Arterial Perfusion	Immersion Fixation	Arterial Perfusion	Immersion Fixation	Immersion Fixation (Series 6)	Arterial Fixation
1.16 ± 0.23 (n = 4)	1.76 ± 0.22* (n = 4)	1.23 ± 0.21 (n = 4)	1.17 ± 0.31 (n = 3)	1.34 ± 0.36 (n = 5)	1.43 ± 0.10 (n = 5)	1.28 ± 0.26 (n = 5)	1.26 ± 0.18 (n = 3)
Capillary Lumen Volume							
0.75 ± 0.07 (n = 4)	0.68 ± 0.22 (n = 4)	0.61 ± 0.09 (n = 4)	0.54 ± 0.17 (n = 3)	0.69 ± 0.24 (n = 5)	0.69 ± 0.07 (n = 5)	0.88 ± 20 (n = 5)	0.72 ± 0.10 (n = 3)

* $P < 0.10$

Values are % of total tissue volume ($\bar{x}$ ± S.E.M.)

TABLE 19

LATTICE PARAMETERS: 1% GLUTARALDEHYDE*

Muscle	IFD (nm)	Ls (μm)	Area ($\mu m^2 \times 10^{-3}$)	Ks ($\mu m^3 \times 10^{-3}$)
Lumbrical	44.7±0.8	2.10±0.010	1.73	3.63
Adductor brevis	42.5±0.6	2.12±0.015	1.56	3.32
Soleus	37.6±0.3	2.32±0.020	1.22	2.84
EDL[†]	36.4±0.5	2.58±0.014	1.15	2.96
Gastrocnemius	41.8±0.6	2.15±0.010	1.51	3.25

*Arterial perfusion of fixative

†Extensor digitorum longus

Values represent $\bar{x}$±SEM of N measurements in each muscle

N = 6 for IFD, N = 30 for Ls, Area = $IFD^2 \cdot \sin 60°$,

Ks = $IFD^2 \cdot \sin 60° \cdot Ls$.

Area and Ks are calculated from the mean of Ls and IFD.

TABLE 20

LATTICE PARAMETERS: 2.5% GLUTARALDEHYDE

Muscle	Method of Fixation	IFD (nm)	Ls (μm)	Area ($\mu m^2 \times 10^{-3}$)	Ks ($\mu m^3 \times 10^{-3}$)
Lumbrical	Arterial Perfusion	48.3±0.5 (n=11)	2.38±0.06	2.02	4.81
		46.8±0.4 (n=6)	2.12±0.02	1.90	4.02
		47.1±1.0 (n=8)	2.04±0.01	1.92	3.92
	$\bar{x}$ =	47.4±0.5 (n=25)	2.18±0.10 (n=90)	1.94±0.04	4.24±0.28 4
	Immersion	47.7±0.3 (n=6)	2.54±0.03	1.97	5.00
		47.3±0.2 (n=8)	2.28±0.09	1.94	4.42
	$\bar{x}$ =	47.5±0.2 (n=14)	2.41±0.21 (n=60)	1.96±0.01	4.71±0.29
Adductor	Arterial Perfusion	48.4±0.4 (n=12)	2.98±0.02	2.03	6.04
		47.5±0.5 (n=9)	3.42±0.04	1.95	6.68
		43.3±0.7 (n=8)	2.59±0.08	1.62	4.21
	$\bar{x}$ =	46.4±1.6 (n=29)	3.00±0.14 (n=90)	1.86±0.12	5.60±0.74
	Immersion	48.3±0.3 (n=8)	1.96±0.07	2.02	3.96
		47.4±0.1 (n=8)	1.98±0.02	1.94	3.85
	$\bar{x}$ =	47.8±0.5 (n=16)	1.97±0.01 (n=60)	1.98±0.04	3.89±0.06

Values expressed as $\bar{x}$±SEM

N=30 for Ls. Area and Ks calculated from the mean of IFD and Ls.

$\bar{x}$ = mean value for each method of fixation.

TABLE 21

VOLUME FRACTION OF MITOCHONDRIA IN LUMBRICAL MUSCLE*

Method of Fixation	AN	AA	AT	IN	IA	IT	SN	SA	ST
Arterial Perfusion	2.14^{3} ±0.79	1.30^{3} ±0.26	1.72^{6} ±0.41	14.90^{3} ±5.18	4.26^{3} ±1.54	9.58^{6} ±3.39	17.04^{3} ±5.59	5.23^{3} ±1.44	11.14^{6} ±3.69
	1.35^{8} ±0.35	0.88^{3} ±0.71	1.22^{11} ±0.39	9.21^{8} ±2.39	6.82^{3} ±3.08	8.56^{11} ±1.43	10.56^{8} ±2.82	7.70^{3} ±3.79	9.78^{11} ±1.69
	2.29^{3} ±0.91	0.12^{3} ±0.10	1.21^{6} ±0.63	9.34^{3} ±1.60	7.02^{3} ±0.39	8.18^{6} ±0.90	11.62^{3} ±2.50	7.14^{3} ±0.26	9.39^{6} ±1.51
$\bar{x}$ =	1.93^{14} ±0.10	0.77^{9} ±0.34	1.38^{23}† ±0.17	11.15^{14} ±1.76	6.03^{9} ±0.89	8.77^{23} ±0.42	13.07^{14} ±1.76	6.69^{9} ±0.75	10.10^{23} ±0.53
Immersion	8.42^{3} ±4.20	0.66^{3} ±0.66	4.54^{6} ±2.51	11.62^{3} ±1.10	10.14^{3} ±2.18	10.88^{6} ±1.14	19.71^{3} ±3.78	10.80^{3} ±2.17	15.26^{6} ±2.79
	2.11^{4} ±0.54	1.35^{4} ±0.37	1.73^{8} ±0.33	5.37^{4} ±0.82	4.79^{4} ±0.55	5.08^{8} ±0.47	7.49^{4} ±0.74	6.14^{4} ±0.40	6.81^{8} ±0.47
$\bar{x}$ =	5.27^{7} ±3.16	1.00^{7} ±0.34	3.14^{14} ±1.40	8.50^{7} ±3.12	7.46^{7} ±2.68	7.98^{14} ±2.90	13.60^{7} ±6.11	8.47^{7} ±2.33	11.03^{14} ±4.22

* Values expressed as $\bar{x}$±SEM% muscle volume.

$\bar{x}$ = mean for each method of fixation.

† $P<0.10$

Superscript numbers = number of measurements.

TABLE 22

VOLUME FRACTION OF MITOCHONDRIA IN ADDUCTOR MUSCLE*

Method of Fixation	AN	AA	AT	IN	IA	IT	SN	SA	ST
Arterial Perfusion	1.38^{4} ±0.55	0.18^{4} ±0.02	0.78^{8} ±0.33	5.43^{4} ±0.53	2.62^{4} ±0.14	4.02^{8} ±0.41	6.54^{4} ±0.79	2.30^{4} ±1.28	4.80^{8} ±1.03
	2.36^{8} ±0.88	0.49^{4} ±0.28	1.74^{12} ±0.95	12.94^{8} ±0.73	8.07^{4} ±2.14	11.32^{12} ±1.27	15.30^{8} ±1.49	8.55^{4} ±2.14	13.05^{12} ±1.84
	1.35^{6} ±0.07	0.37^{3} ±0.18	1.02^{9} ±0.24	15.60^{6} ±2.42	11.85^{3} ±2.46	14.35^{9} ±1.33	16.95^{6} ±2.42	12.88^{3} ±2.17	15.59^{9} ±1.29
$\bar{x}$ =	1.70^{18} ±0.33	0.35^{11}† ±0.09	1.18^{29} ±0.29	11.32^{18} ±3.04	7.51^{11} ±2.68	9.90^{29} ±3.07	12.93^{18} ±3.23	7.91^{11} ±3.07	11.15^{29} ±3.26
Immersion	3.06^{4} ±1.15	1.32^{4} ±0.78	2.19^{8} ±0.72	10.30^{4} ±0.66	12.14^{4} ±1.67	11.22^{8} ±0.90	13.35^{4} ±1.77	13.46^{4} ±1.64	13.41^{8} ±1.12
	1.29^{4} ±0.33	0.87^{4} ±0.20	1.08^{8} ±0.20	10.73^{4} ±0.74	7.54^{4} ±0.76	8.89^{8} ±0.70	11.53^{4} ±0.93	8.40^{4} ±0.90	9.97^{8} ±0.84
$\bar{x}$ =	2.18^{8} ±0.88	1.10^{8} ±0.22	1.64^{16} ±0.56	10.52^{8} ±0.22	9.84^{8} ±2.30	10.06^{16} ±1.16	12.44^{8} ±0.57	10.93^{8} ±2.53	11.69^{16} ±1.72

* Values expressed as $\bar{x}$±SEM% muscle volume.

$\bar{x}$ = mean for each method of fixation

†$P<0.05$

Superscript numbers = number of measurements

TABLE 23

RELATIVE POSITIONS OF MITOCHONDRIA IN A CELL

Method of Fixation	Position in Cell	Adductors		Lumbricals	
		I-band	A-band	I-band	A-band
Arterial Perfusion	Total	90%	10%	87%	13%
	Near*	88%	12%	85%	15%
	Center	93%	7%	88%	12%
Immersion	Total	85%	15%	71%	29%
	Near*	83%	17%	58%	42%
	Center	90%	10%	94%	6%

* Near = adjacent to a capillary

TABLE 24

VOLUME FRACTION OF SARCOPLASMIC RETICULUM IN LUMBRICAL MUSCLE*

Method of Fixation	AN	AA	AT	IN	IA	IT	SN	SA	ST
Arterial Perfusion	2.62^{3} ±0.58	2.39^{3} ±0.44	2.50^{6} ±0.33	3.49^{3} ±0.51	4.53^{3} ±1.15	4.01^{6} ±0.61	6.11^{3} ±1.06	6.59^{3} ±1.59	6.35^{6} ±0.86
	2.92^{8} ±0.25	3.03^{3} ±0.09	3.04^{11} ±0.16	4.38^{8} ±0.38	4.47^{3} ±0.24	4.41^{11} ±0.17	7.31^{8} ±0.50	7.50^{3} ±0.33	7.36^{11} ±0.25
	3.46^{3} ±0.14	3.00^{3} ±0.26	3.23^{6} ±0.17	4.46^{3} ±0.35	4.60^{3} ±0.42	4.53^{6} ±0.24	7.92^{3} ±0.53	7.60^{3} ±0.55	7.76^{6} ±0.35
$\bar{x}$ =	3.00^{14} ±0.24	2.81^{9} ±0.21	2.92^{23} ±0.22	4.08^{14} ±0.30	4.53^{9} ±0.04	4.32^{23} ±0.16	7.09^{14} ±0.53	7.23^{9} ±0.32	7.16^{23} ±0.42
Immersion	2.39^{3} ±0.49	2.51^{3} ±0.37	2.45^{6} ±0.28	3.63^{3} ±0.84	4.33^{3} ±0.44	3.98^{6} ±0.45	6.02^{3} ±1.32	6.84^{3} ±0.75	6.43^{6} ±0.70
	3.66^{4} ±0.20	2.72^{4} ±0.35	3.19^{8} ±0.24	6.66^{4} ±0.96	6.17^{4} ±0.46	6.42^{8} ±0.50	9.39^{4} ±0.22	8.89^{4} ±0.48	9.14^{8} ±0.26
$\bar{x}$ =	3.03^{7} ±0.64	2.62^{7} ±0.10	2.82^{14} ±0.37	5.14^{7} ±1.52	5.25^{7} ±0.92	5.20^{14} ±1.22	7.70^{7} ±1.68	7.86^{7} ±1.02	7.78^{14} ±1.36

* Values expressed as $\bar{x}$±SEM% muscle volume.

$\bar{x}$ = mean for each method of fixation.

Superscript numbers = number of measurements.

TABLE 25

VOLUME FRACTION OF SARCOPLASMIC RETICULUM IN ADDUCTOR MUSCLE*

Method of Fixation	AN	AA	AT	IN	IA	IT	SN	SA	ST
Arterial Perfusion	2.69^{4} ±0.10	2.72^{4} ±0.28	2.70^{8} ±0.14	5.84^{4} ±0.14	6.59^{4} ±0.01	6.22^{8} ±0.26	8.53^{4} ±0.48	9.26^{4} ±0.22	8.89^{8} ±0.28
	2.81^{8} ±0.21	2.64^{4} ±0.35	2.75^{12} ±0.14	4.15^{8} ±0.36	4.74^{4} ±0.59	4.35^{12} ±0.29	6.96^{8} ±0.60	7.38^{4} ±0.87	7.10^{12} ±0.39
	1.65^{6} ±0.31	2.22^{3} ±0.17	1.84^{9} ±0.16	3.71^{6} ±0.35	4.31^{3} ±0.16	3.91^{9} ±0.16	5.74^{6} ±0.33	5.63^{3} ±0.41	5.70^{9} ±0.24
$\bar{x}$ =	2.38^{18} ±0.42	2.53^{11} ±0.16	2.42^{29} ±0.28	4.51^{18} ±0.66	5.21^{11} ±0.70	4.83^{29} ±0.68	7.08^{18} ±0.78	7.42^{11} ±1.08	7.23^{29} ±0.91
Immersion	2.69^{4} ±0.16	2.01^{4} ±0.36	2.35^{8} ±0.20	4.41^{4} ±0.80	5.50^{4} ±0.85	4.95^{8} ±0.57	7.09^{4} ±0.78	7.51^{4} ±0.88	7.30^{8} ±0.55
	2.28^{4} ±0.37	2.24^{4} ±0.44	2.26^{8} ±0.27	4.00^{4} ±0.35	3.66^{4} ±0.10	3.83^{8} ±0.17	6.28^{4} ±0.71	5.90^{4} ±0.39	6.10^{8} ±0.39
$\bar{x}$ =	2.49^{8} ±0.20	2.13^{8} ±0.12	2.31^{16} ±0.04	4.20^{8} ±0.20	4.58^{8} ±0.92	4.39^{16} ±0.56	6.68^{8} ±0.40	6.70^{8} ±1.14	6.70^{16} ±0.60

* Values expressed as $\bar{x}$±SEM% muscle volume.

$\bar{x}$ = mean value for each method of fixation.

Superscript numbers = number of measurements.

TABLE 26

CHANGE IN CELL AND ORGANELLE VOLUMES FOLLOWING ACUTE HEMORRHAGE

Series	ΔIFD (% cell volume)	ΔLs (%)	ΔKs (%)	ΔVM (% mitochondria volume) (% cell volume)	ΔVSR (%SR volume) (% cell volume)
I	↑6.3*	↓1.90†	↑11	↓43.2* (↓3.66)	↓63.3§ (↓6.75)
II	↓1.9	↑20.6§	↑16	↓20.7 (↓2.3)	↑19.8 (↑1.3)
III	↓5.4*	↓17.3§	↓26	↓48.8* (↓7.45)	↑40.2† (↑2.59)
IV	↑0.8	↓12.4§	↓11	↓41 (↓1.97)	↓35.1§ (↓3.12)
V	↓3.4¶	↑59.0§	↑39	↓6.3 (↓0.8)	↓18.5† (↓1.35)
VI	↑2.3§	↑9.1§	↑49	↑3.0 (↑0.3)	↑26.5* (↑1.6)

*P<0.10
†P<0.05
¶P<0.01
§P<0.001

TABLE 27

CORRELATION COEFFICIENTS

Series	VM						VSR					
	I	II	III	IV	V	VI	I	II	III	IV	V	VI
IFD	-0.48	0.49	0.60	-0.13	0.36	0.27	-0.30	-0.31	-0.55	-0.55	-0.58	0.21
Ls	0.54	0.09	0.49	0.86*	0.48	0.03	0.12	-0.27	-0.45	0.02	-0.58	-0.22
VI	0.22	0.07	0.84†¶	0.91*¶	0.61	0.22	0.42	-0.16	-0.61	0.13	0.30	0.65

*P<0.01

†P<0.05

¶Positive correlation due to high correlation between VM adjacent to the sarcolemma (VM-SN) and VI (Series III, 5=0.92, P<0.01; Series IV, r=0.94, P<0.01).

FIGURES

Figure 1

Stages of shock (Shoemaker, 1967).

A - control period
B_1 - decompensation
B_2 - stabilization
C - resuscitation
D - recovery
E - preterminal

Pathogenic mechanism: Stages A, B_1, B_2.
Modifiers of pathogenic mechanisms: Stages C, D.
Terminal mechanisms: Stage E.

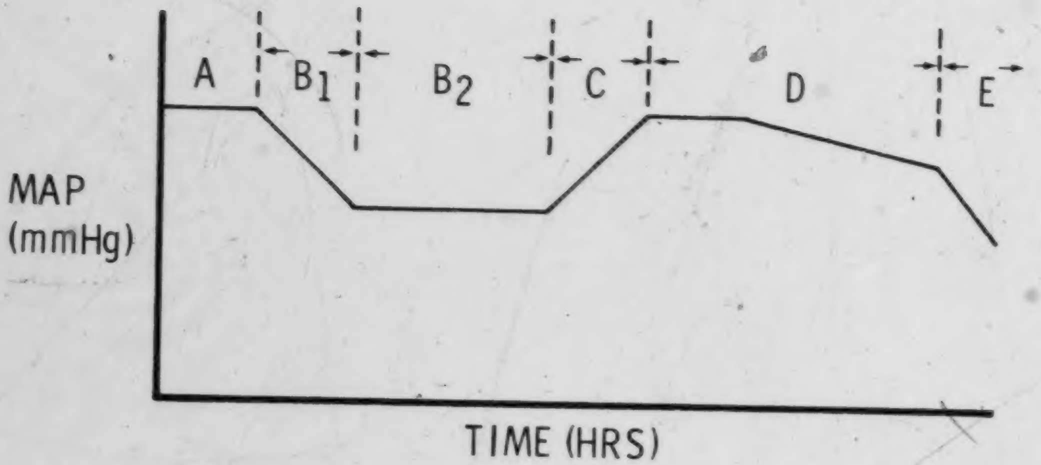

A
B_1
B_2
C
D
E
MAP
(mmHg)
TIME (HRS)

Figure 2

Body fluid compartments expressed as a percentage of total body water. 'Functional' extracellular fluid (27.5%) includes interstitial space volume (20%) and plasma water (7.5%). Values are based on 'average' determination by various methods of analysis (Cizek, 1968).

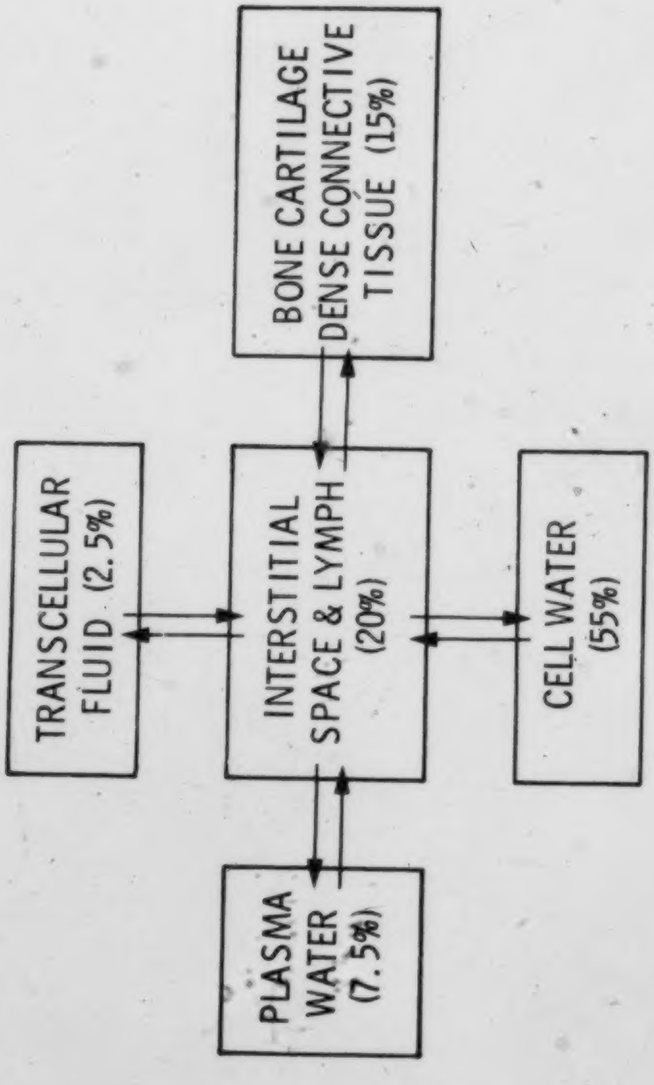

TRANSCELLULAR FLUID (2.5%)
INTERSTITIAL SPACE & LYMPH (20%)
CELL WATER (55%)
BONE CARTILAGE DENSE CONNECTIVE TISSUE (15%)
PLASMA WATER (7.5%)

Figure 3

Schematic representation of the ultrastructure of skeletal muscle (from Peachey, 1965b). In rat, the terminal cisternae occur at the A-I junction and the H-zone sacs are not as well delineated as in the illustration.

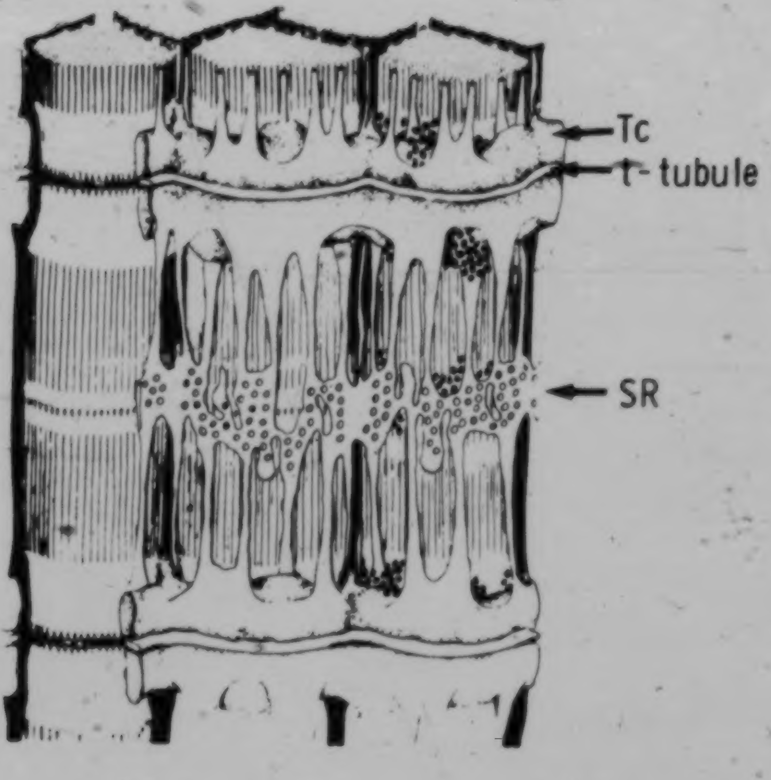
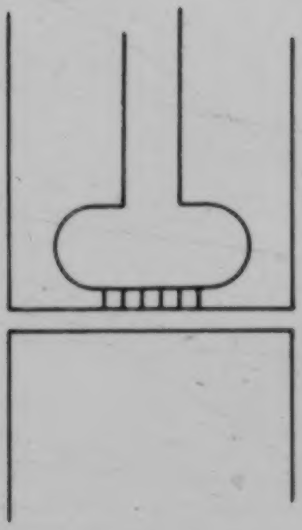

Tc
t- tubule
SR

Figure 4

Proposed relationship between SR sacs and t-tubules.

(a) Non-anatomic, electrical coupling concept (Franzini-Armstrong, 1971).

(b) Open pore direct continuity proposed by Birks (1965) involving direct continuity between the extracellular space and the SR.



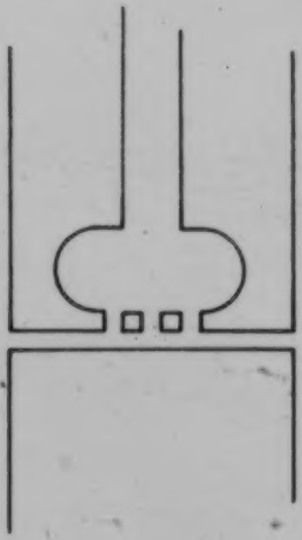


Figure 5

Response of mean arterial pressure to intraperitoneal pentobarbital (20 mg/kg) and methoxyflurane (Penthrane) inhalation. Note stability of mean arterial pressure with methoxyglurane anesthesia. Rhythmic fluctuations reflect respiration.

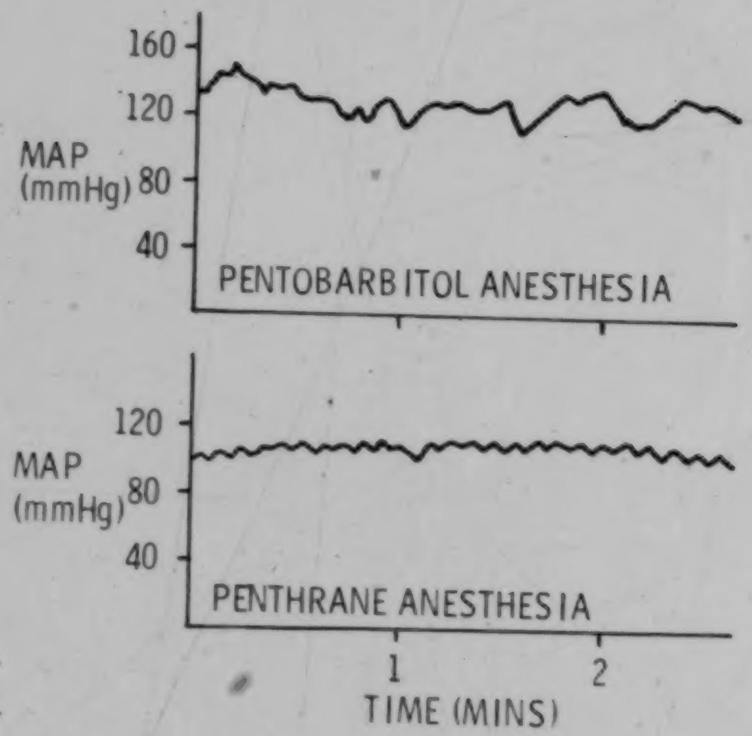

160
120
MAP
(mmHg)
80
40
PENTOBARBITOL ANESTHESIA
120
MAP
(mmHg)
80
40
PENTHRANE ANESTHESIA
1
2
TIME (MINS)

Figure 6

Nose cone method of maintaining methoxyflurane anesthesia.

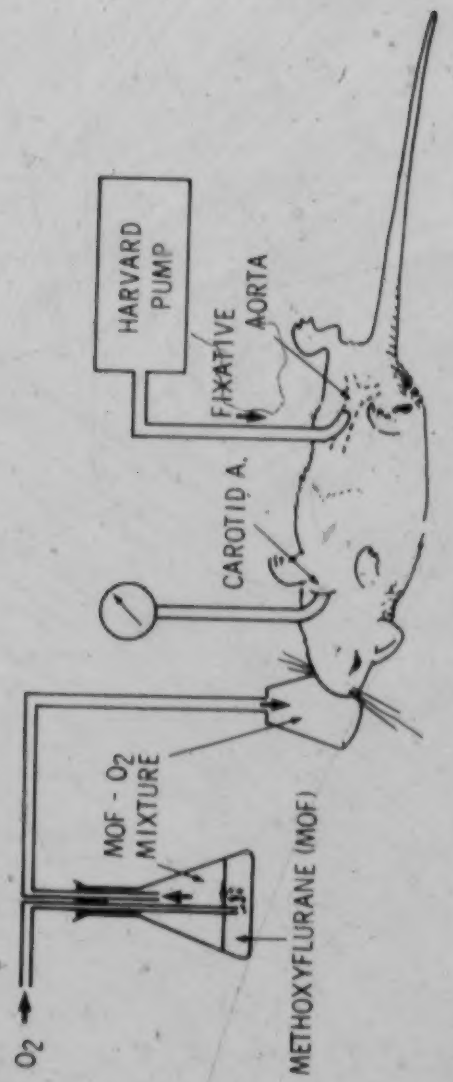

HARVARD PUMP
FIXATIVE
AORTA
CAROTID A.
MOF - O_2 MIXTURE
METHOXYFLURANE (MOF)
O_2

Figure 7

Effect of flushing arterial cannula to maintain patency. No significant effect on arterial pressure is seen.

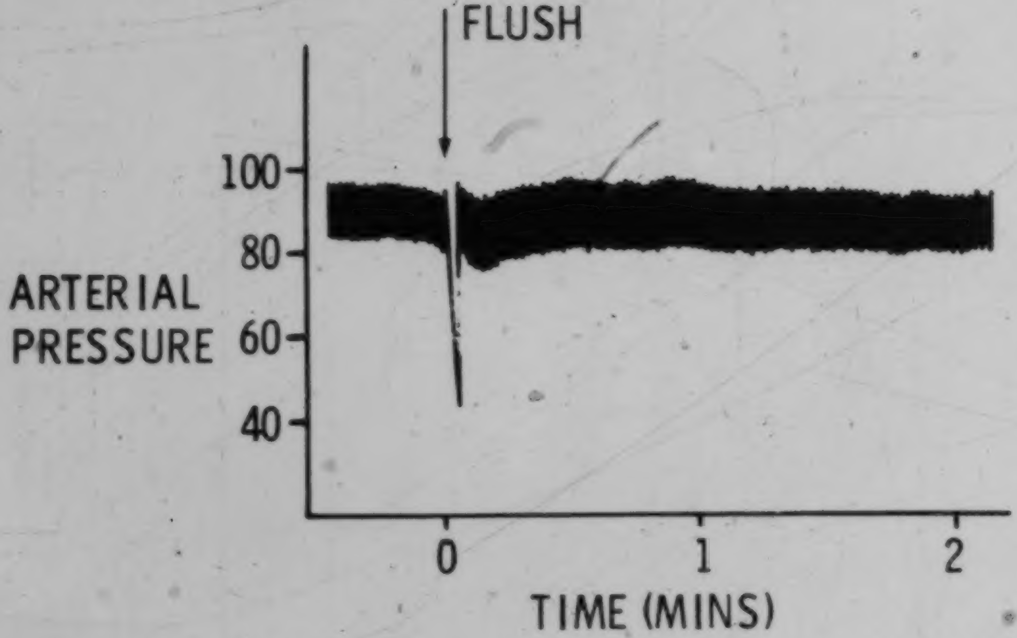

FLUSH
ARTERIAL PRESSURE
100
80
60
40
0
1
2
TIME (MINS)

Figure 8

Schematic representation of procedure during hemorrhage. Biopsies (B) are taken at predetermined arterial pressures 1-2 minutes after a small hemorrhage (vertical arrows) when the pressure is partially stabilized. Total blood loss equals 40% of estimated blood volume.

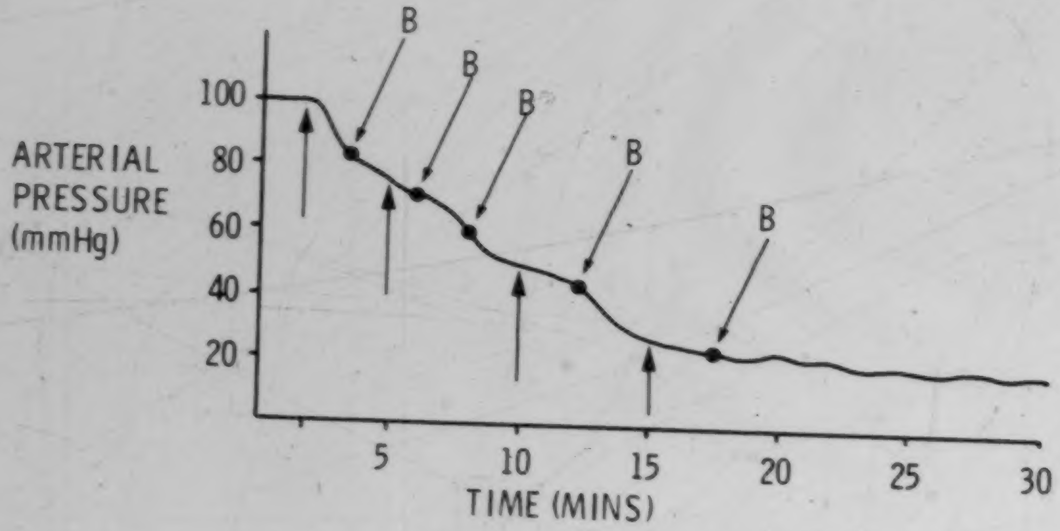

ARTERIAL PRESSURE (mmHg)
100
80
60
40
20
B
B
B
B
B
5
10
15
20
25
30
TIME (MINS)

Figure 9

Pressure (mm Hg) obtained from the aortic cannula during perfusion of fixative with a Harvard constant volume infusion pump. Fluctuations in pressure are seen almost immediately after perfusion is started and closely resemble the effect of respiration on arterial pressure.

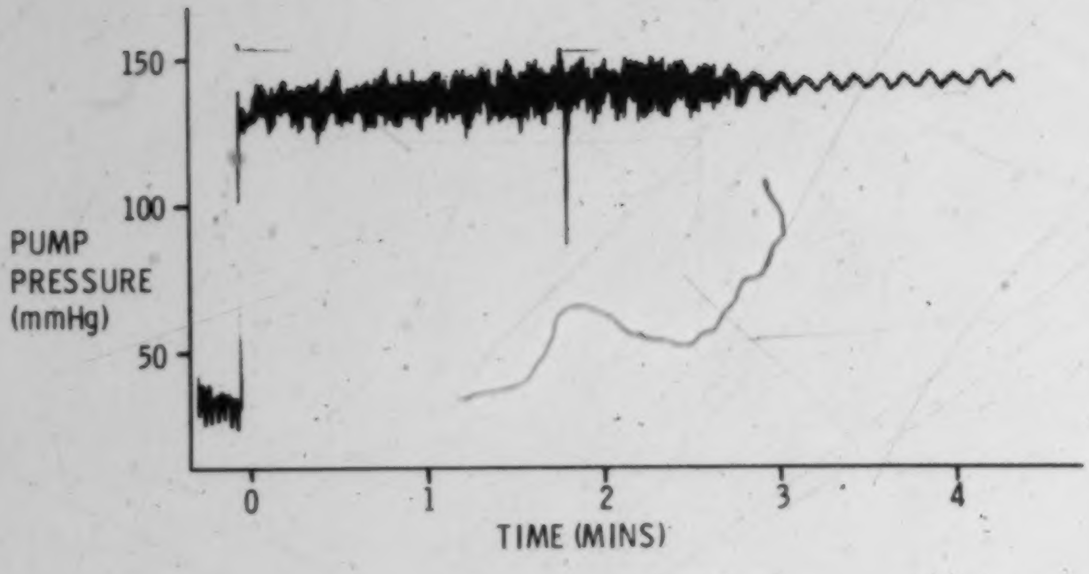

PUMP
PRESSURE
(mmHg)
150
100
50
0
1
2
3
4
TIME (MINS)

Figure 10

Specimen orientation. A is used for longitudinal sections. B is used for transverse segments.

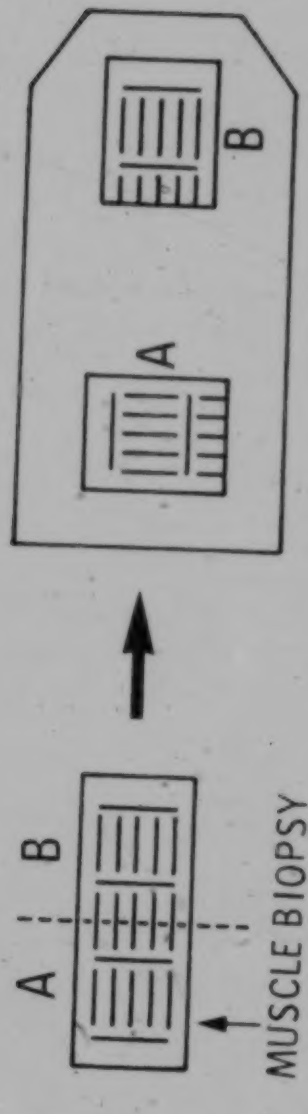

A
B
MUSCLE BIOPSY
A
B

Figure 11

Regular point lattice used for estimating the volume fraction of a component i within a tissue.

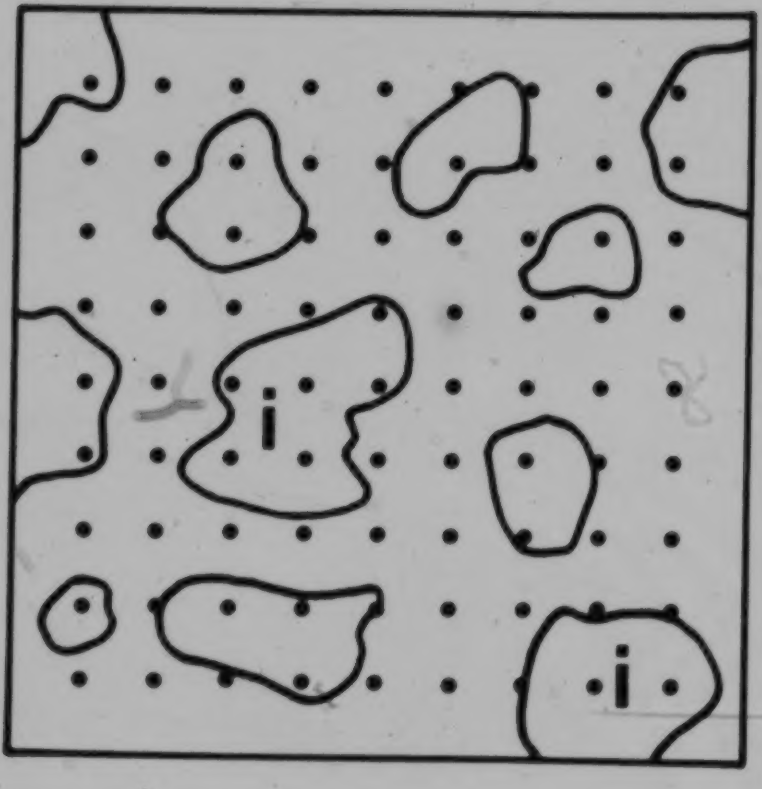

i
i

Figure 1[illegible]

Simple regular square lattice grid for estimating volumes of large components.

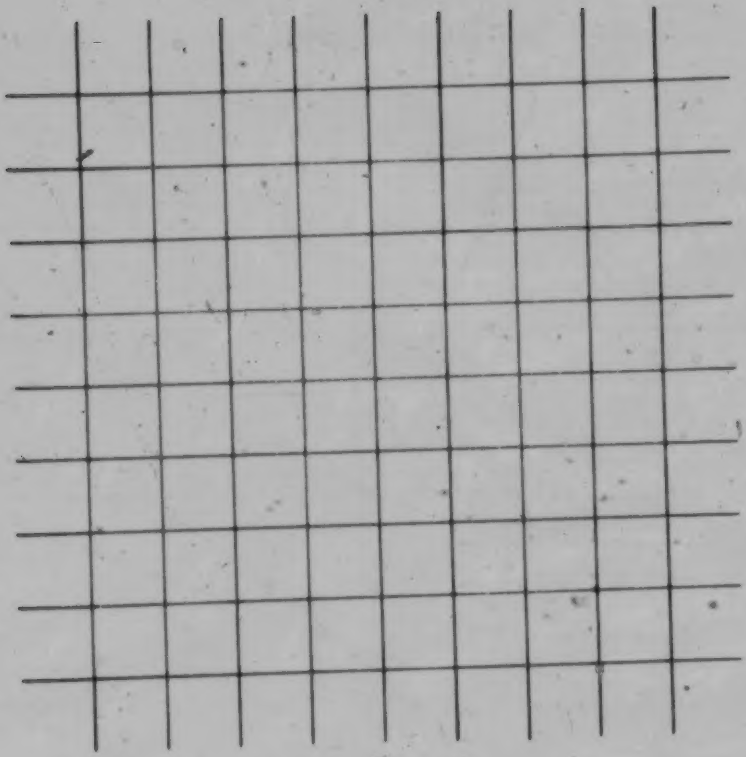


Figure 13

Double lattice grid for estimating volumes of small components.

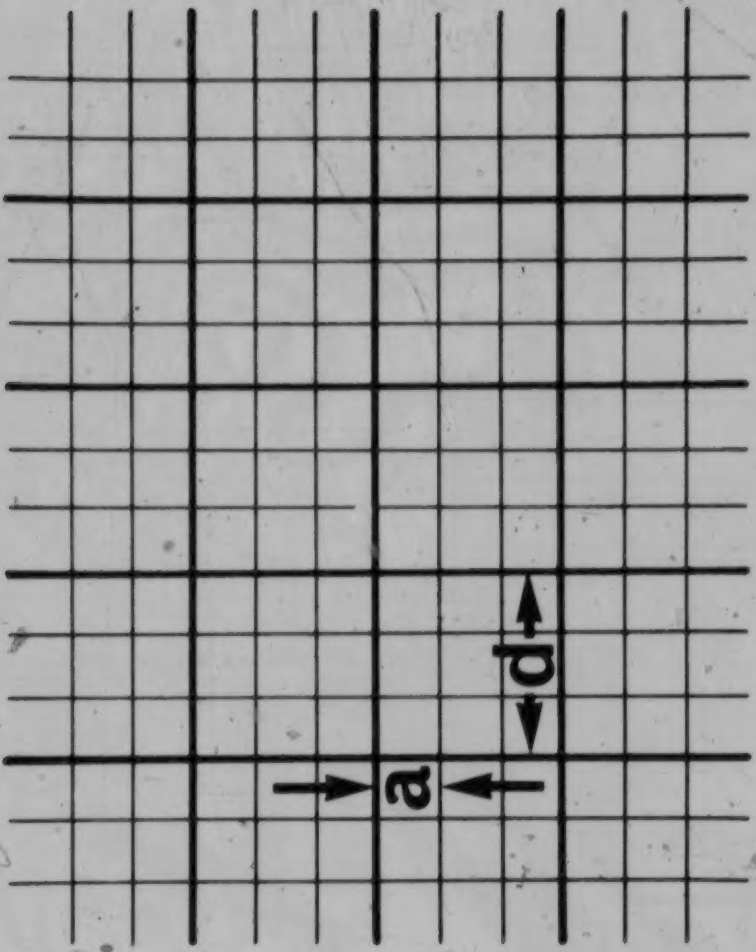

a
d

Figure 14

Capillary volume in lumbricals fixed by arterial perfusion of 2.5% glutaraldehyde. Note that the volume of the capillary lumen does not change to the same degree that total capillary volume changes. Both VC and VL decrease significantly ($P<0.01$) with the initial drop in arterial pressure to 80 mm Hg. Subsequently both VC and VL return to normal. ($^*P<0.01$; $^\dagger P<0.10$)

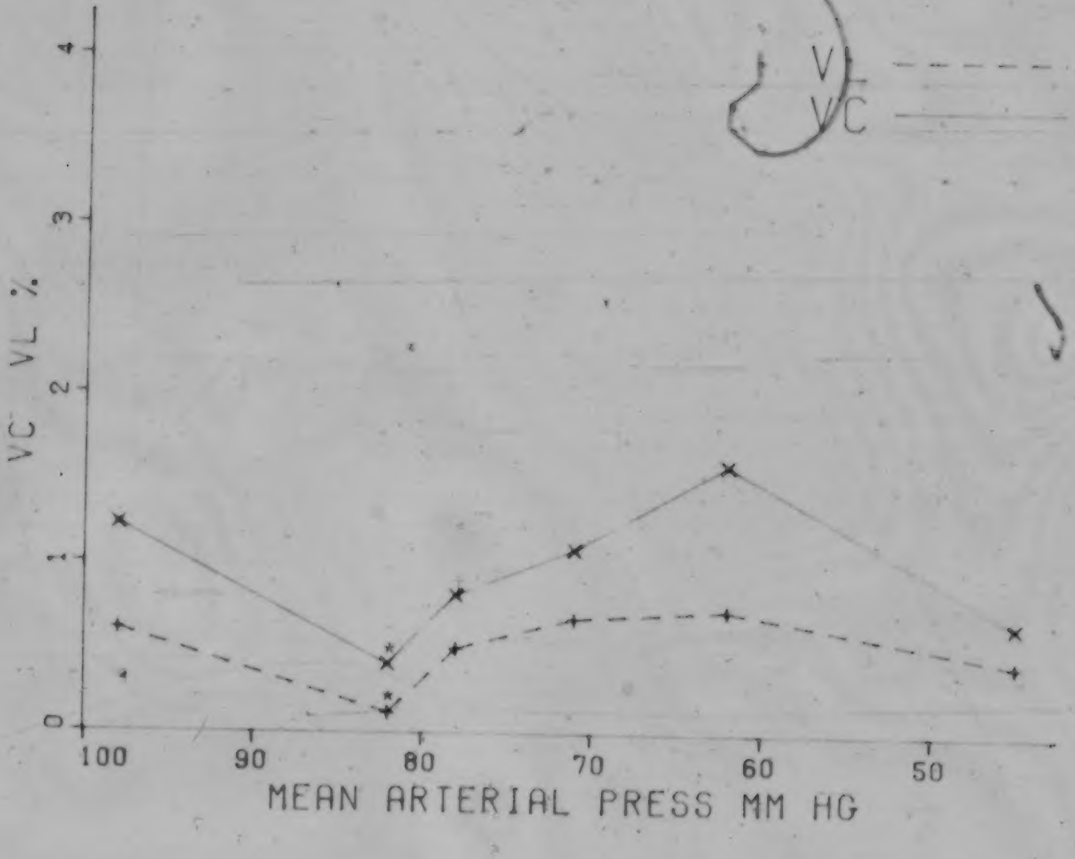

VL
VC
VC VL %
0
1
2
3
4
100
90
80
70
60
50
MEAN ARTERIAL PRESS MM HG

Figure 15

Capillary volume in lumbricals fixed by immersion in 2.5% glutaraldehyde. The discrepancy between changes in total capillary volume and lumen volume is again evident. However, as for lumbricals fixed by arterial perfusion of 2.5% glutaraldehyde (Fig. 14) there is a significant decrease ($P<0.10$) in VL and VC with the initial drop in arterial pressure.

($^*P<0.10$)

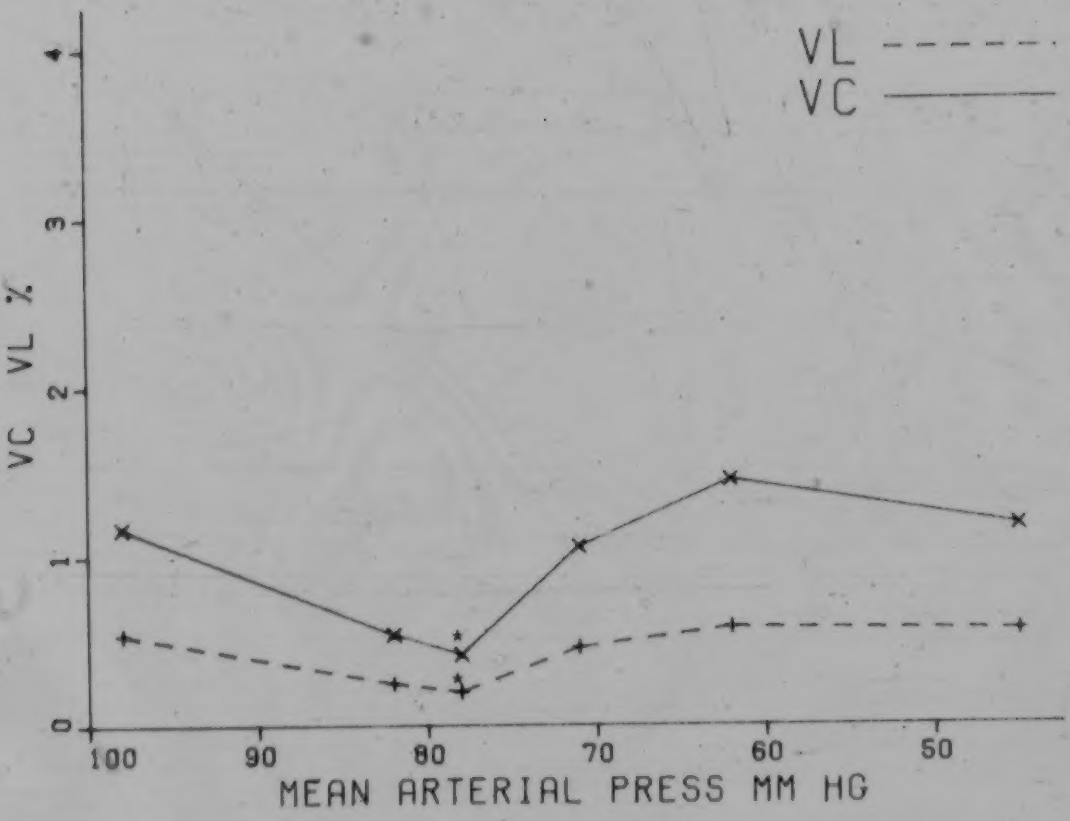

VL
VC
VC VL %
0
1
2
3
4
100
90
80
70
60
50
MEAN ARTERIAL PRESS MM HG
*
*

Figure 16

Capillary volume: adductor brevis fixed by immersion in 2.5% glutaraldehyde. The variation in total and lumen volumes is less apparent than with arterial fixation. A significant decrease in V_C ($P<0.01$) and V_L ($P<0.10$) is again evident following the initial hemorrhage. (*$P<0.01$; †$P<0.10$)

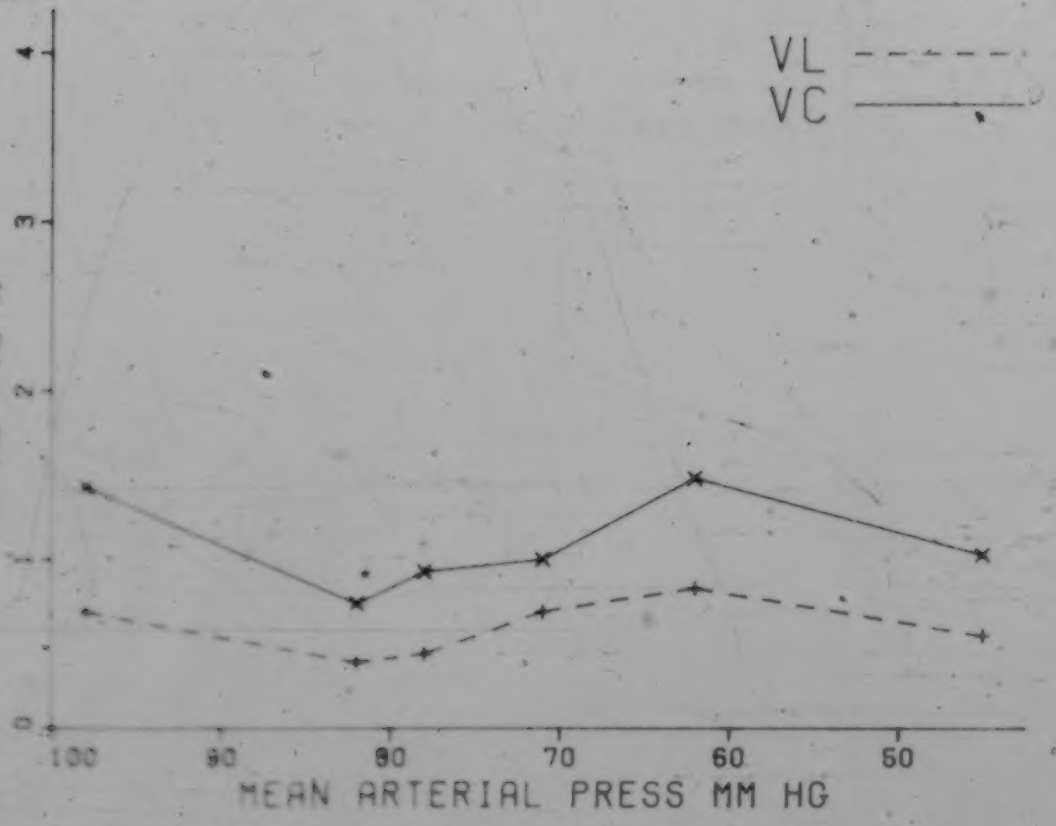

VL
VC
VC VL %
0
1
2
3
4
100
90
80
70
60
50
MEAN ARTERIAL PRESS MM HG

Figure 17

Capillary volume in adductor brevis fixed by perfusion of 2.5% glutaraldehyde. Total volume (VC) changes more than lumen volume (VL). For example at 82 mm Hg, VC decreases by 0.80% ($P<0.05$) but VL decreases by only 0.61%, ($P<0.05$); the decrease in VC is greater than the normal VL. Consequently some change in the endothelial cell must occur, either a decrease in cytoplasmic volume or a decrease in size or number of vesicles. There is a second reduction in VC and VL at 45 mm Hg ($P<0.10$). (*$P<0.05$; †$P<0.10$)

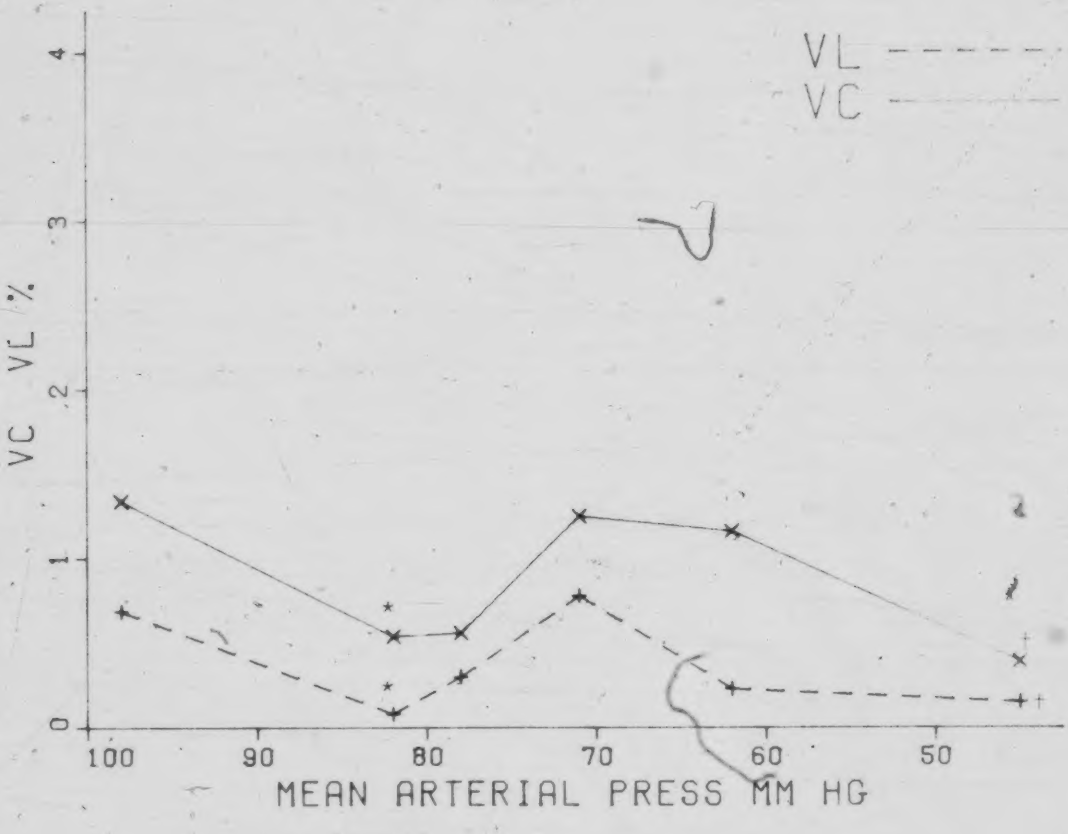

VL
VC
VC VL %
0
1
2
3
4
100
90
80
70
60
50
MEAN ARTERIAL PRESS MM HG

Figure 18

Capillary volume. Lumbrical muscles fixed by carpal tunnel perfusion of 1% glutaraldehyde. The pattern of response is very different from lumbricals fixed by arterial perfusion or by 2.5% glutaraldehyde. ($*P<0.10$)

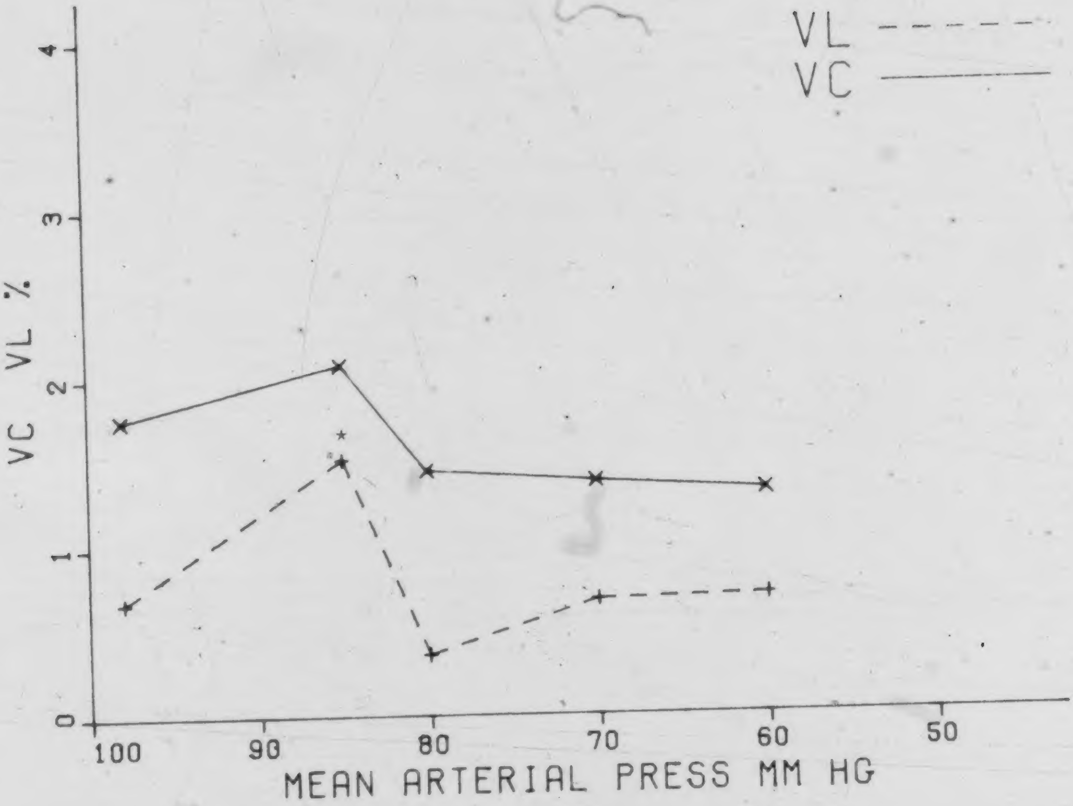

VL
VC
VC VL %
0
1
2
3
4
*
100
90
80
70
60
50
MEAN ARTERIAL PRESS MM HG

Figure 19

Capillary volume.* Lumbrical muscles perfused with 1% glutaraldehyde. Lumen volume (VL) is constant. However only VC at 60 mm Hg is significantly greater than normal VC ($P<0.10$). Consequently either the endothelial cytoplasm has increased in volume or the volume and number of vesicles have significantly increased. (*$P<0.10$)

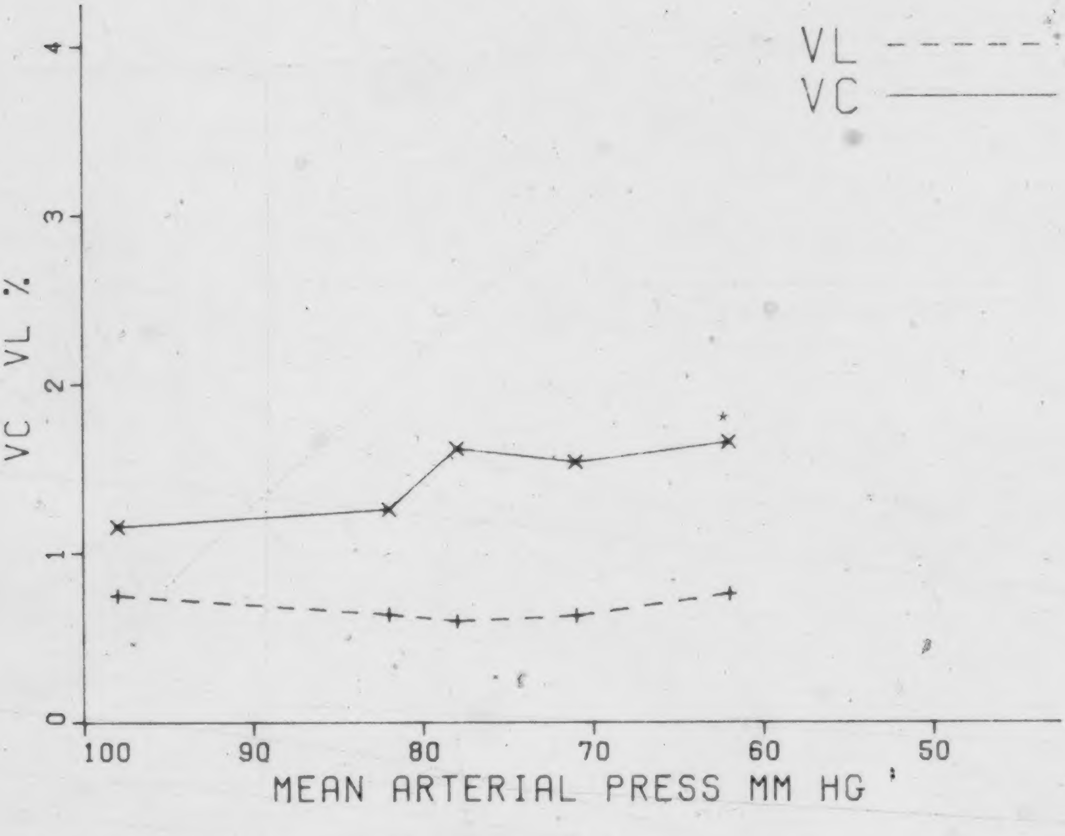

VL
VC
VC VL %
0
1
2
3
4
100
90
80
70
60
50
MEAN ARTERIAL PRESS MM HG
*

Figure 20

Capillary volume. Adductor muscles fixed by immersion in 2.5% glutaraldehyde. Note that the response of total and lumen volumes is the same as in the other series until M.A.P. is 50 mm Hg. Total volume (VC) and lumen volume (VL) at 30 mm Hg are normal. (*$P<0.05$)

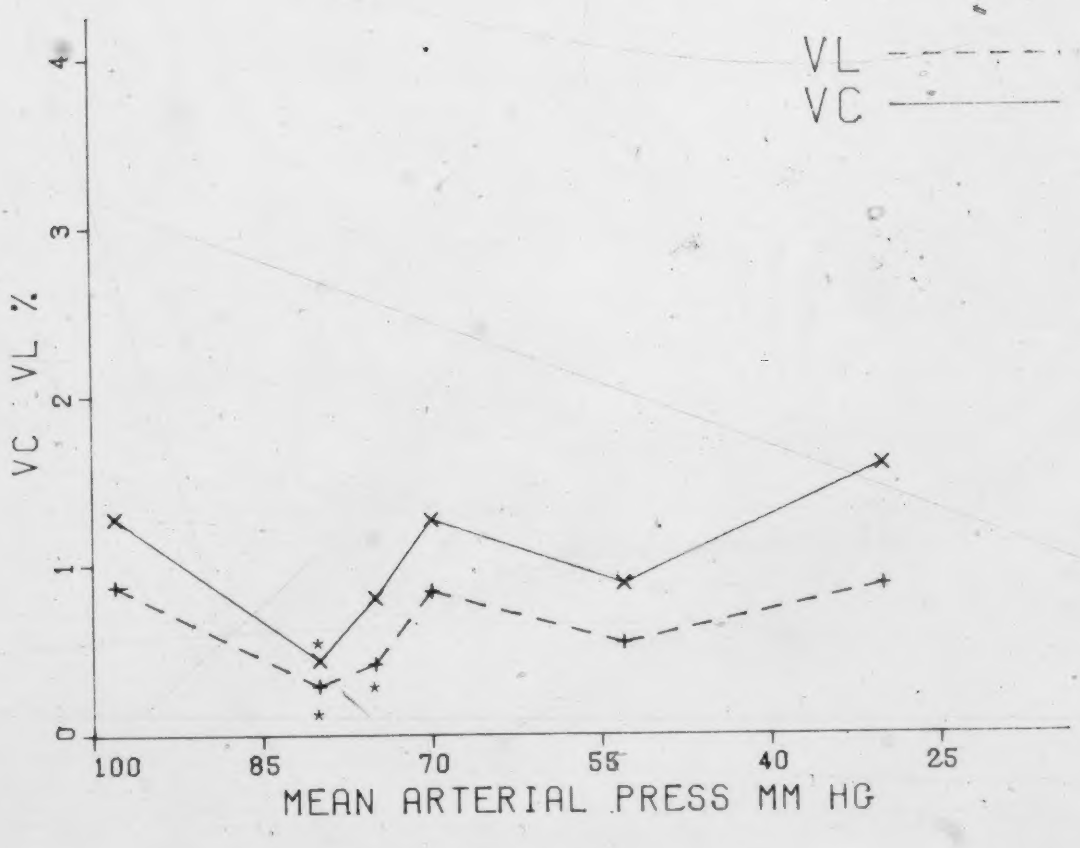

VL
VC
VC : VL %
0
1
2
3
4
100
85
70
55
40
25
MEAN ARTERIAL PRESS MM HG
*
*
*

Figure 21

Schematic representation of branching capillary. A section through the junction might yield the impression of an intralumenal extension of endothelium.

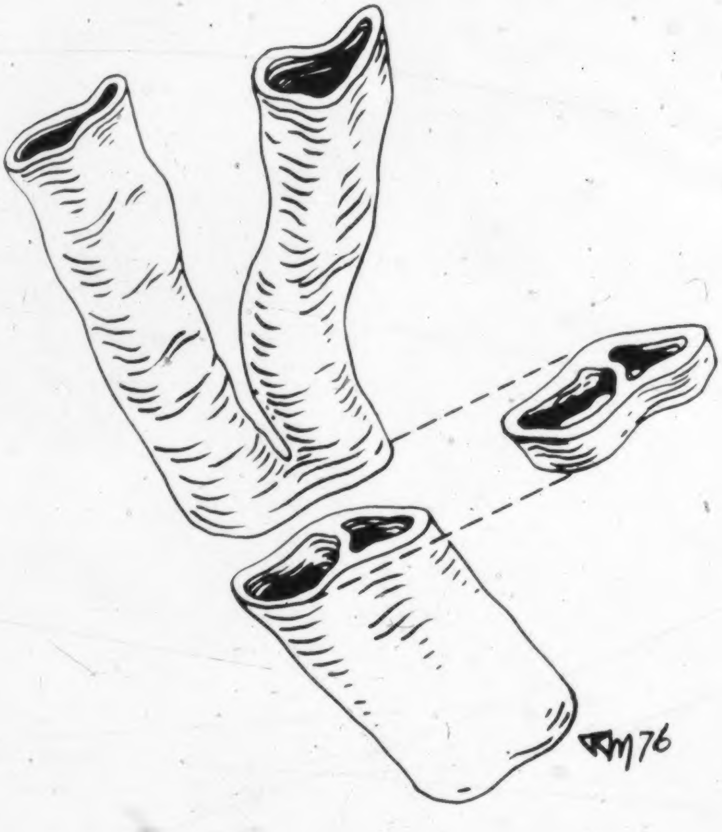

RM76

Figure 22

Schematic representation of the rat skeletal muscle myofilament array. The circles represent thick (myosin) filaments. The dots represent the thin (actin) filaments. The spacing d represents the $\bar{1},\bar{0}$ lattice plane (April, *et al*, 1971) and is equal to IFD · sin 60°. The heavy solid lines define the unit cell. The unit cell area $A = IFD^2 \cdot \sin 60°$. IFD = distance between thick filaments, D.

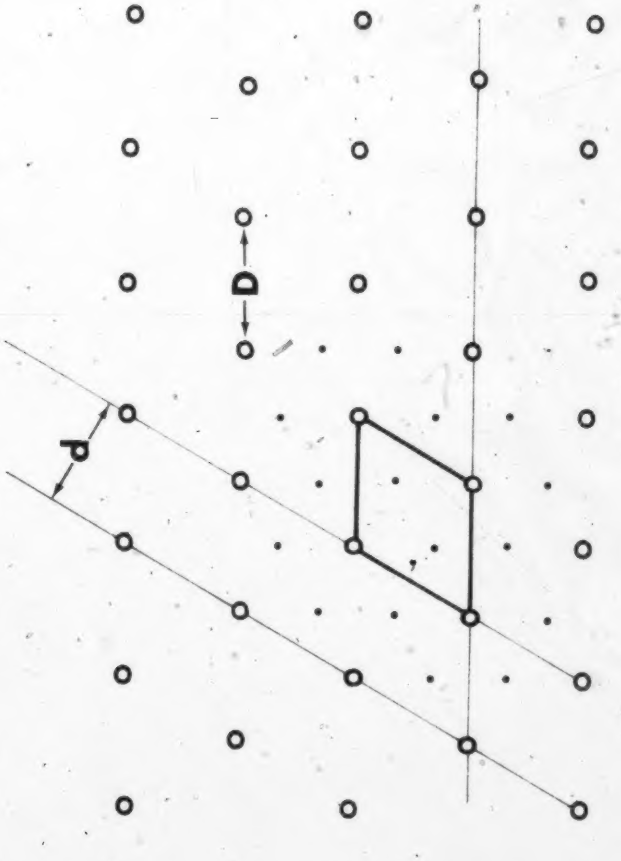

D
d

Figure 23

Series I - Cell volume (FD) (=IFD) and unit cell volume (SK) (=Ks) response to hemorrhage. IFD decreases ($P<0.01$) with the initial drop in arterial pressure. This is followed by an increase to normal at 60 mm Hg. ($P>0.05$). Sarcomere length (SL) (=Ls) follows an opposite pattern. It initially increases significantly ($P<0.01$) but at 60 mm Hg it is significantly decreased ($P<0.05$). Note that when SL is greatest capillary lumen volume (Figure 19) is least. Lumbrical muscles; 1% glutaraldehyde arterial perfusion

(§$P<0.001$; †$P<0.05$; ¶$P<0.01$; *$P<0.10$)

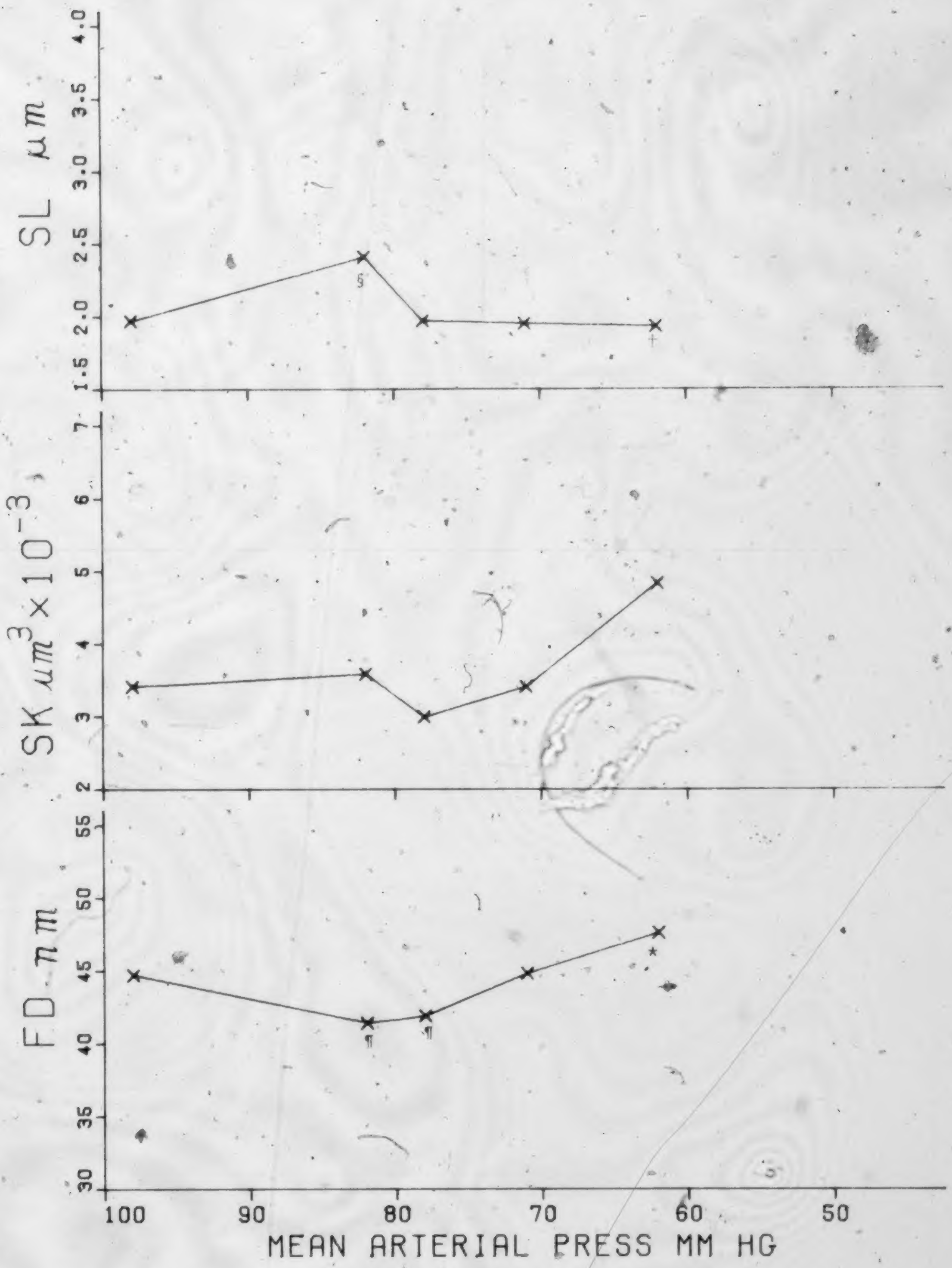

SL µm
1.5
2.0
2.5
3.0
3.5
4.0
§
†
SK µm³ × 10⁻³
2
3
4
5
6
7
FD nm
30
35
40
45
50
55
¶
¶
*
100
90
80
70
60
50
MEAN ARTERIAL PRESS MM HG

Figure 24

Series II - Cell volume (FD) and sarcomere length both show variable response to hemorrhage. Final values for IFD, Ls and Ks are within normal range. Sarcomere length again is greatest when capillary lumen volume (pressure) is least. This implies extreme sensitivity of the contractile components to changes in capillary pressure. Lumbricals; 2.5% glutaraldehyde; arterial perfusion.

(¶$P<0.001$; *$P<0.10$; †$P<0.05$)

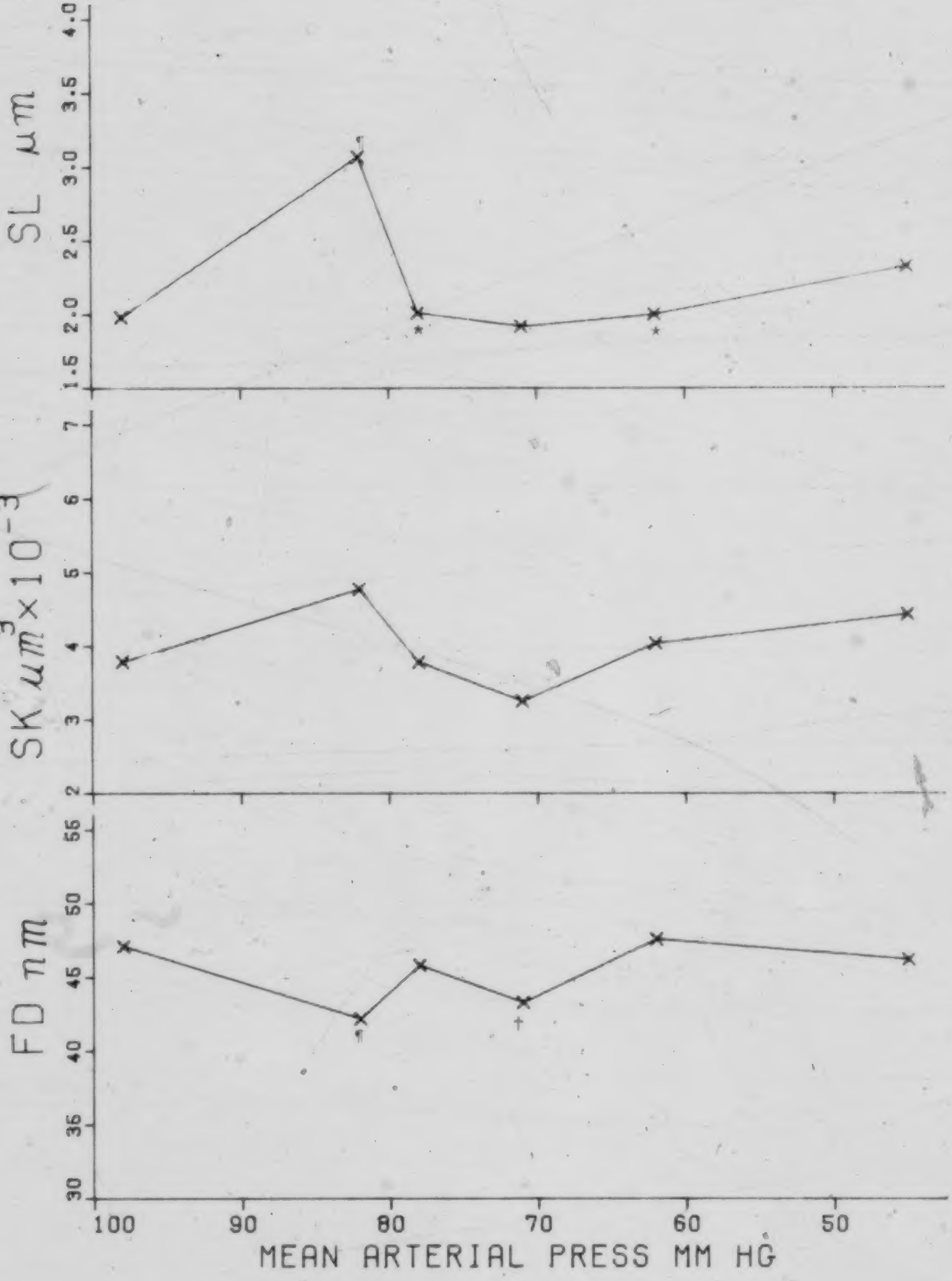

SL μm
1.5
2.0
2.5
3.0
3.5
4.0
*
*
SK μm³×10⁻³
2
3
4
5
6
7
FD nm
30
35
40
45
50
55
¶
†
100
90
80
70
60
50
MEAN ARTERIAL PRESS MM HG

Figure 25

Series III - Lumbrical muscles; immersion fixation with 2.5 glutaraldehyde. Note lack of variation in IFD in contrast to lumbricals fixed by arterial perfusion (Figure 24). Ls is decreased significantly ($P<0.01$) from 80 mm Hg to 45 mm Hg. IFD remains normal.

(*$P<0.001$; †$P<0.10$)

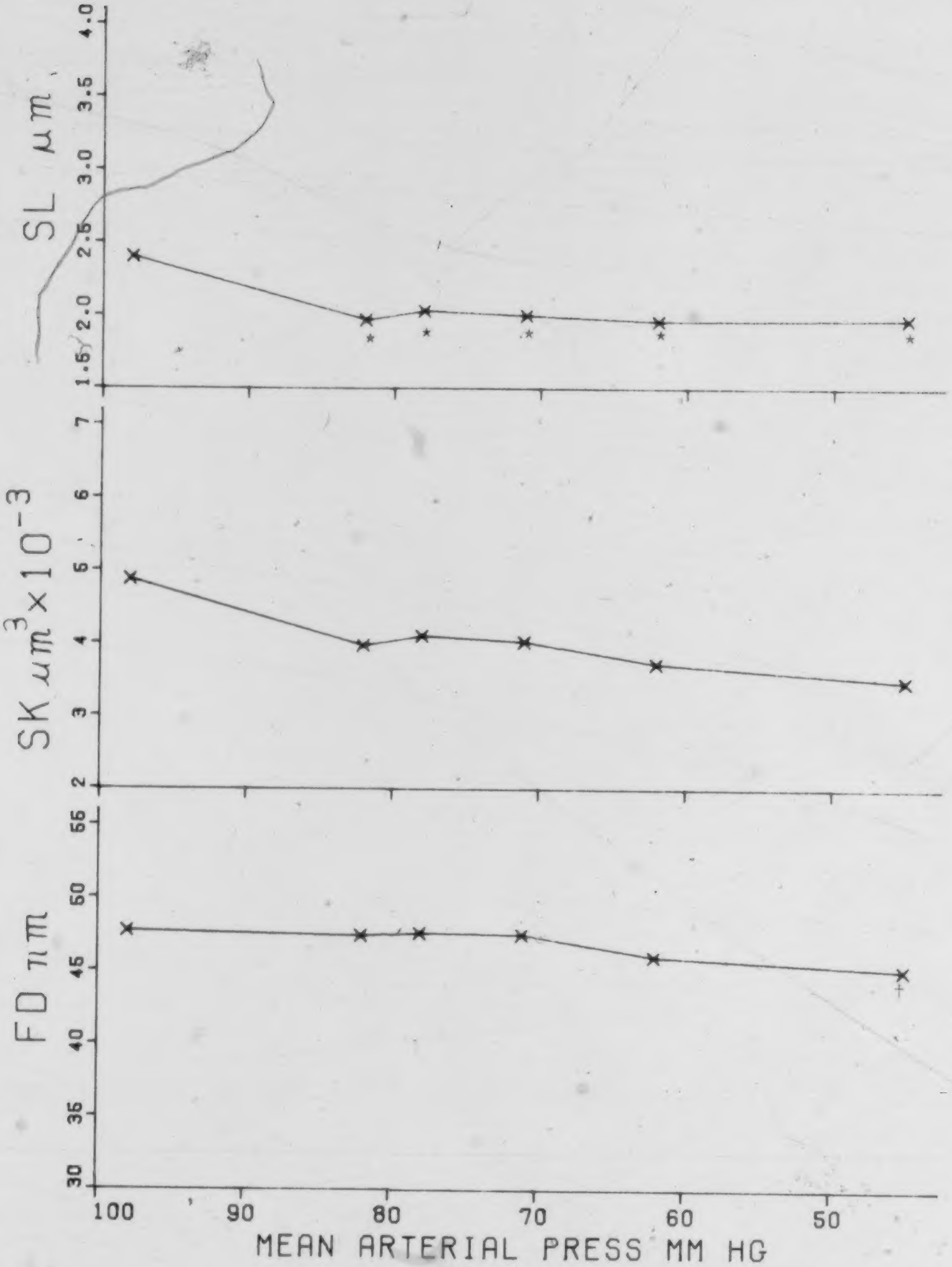

SL μm
4.0
3.5
3.0
2.5
2.0
1.5
*
SK $\mu m^3 \times 10^{-3}$
7
6
5
4
3
2
FD nm
55
50
45
40
35
30
+
100
90
80
70
60
50
MEAN ARTERIAL PRESS MM HG

Figure 26

Series IV - Adductor muscle; 2.5% glutaraldehyde; arterial perfusion fixation. No change in cell water occurs. Sarcomere length increases ($P<0.01$) following the initial drop in capillary pressure, again demonstrating the initial sensitive relationship of contraction and capillary flow. At 45 mm Hg MAP however, sarcomere length is decreased ($P<0.01$) but IFD is normal.

(*$P<0.01$; +$P<0.001$)

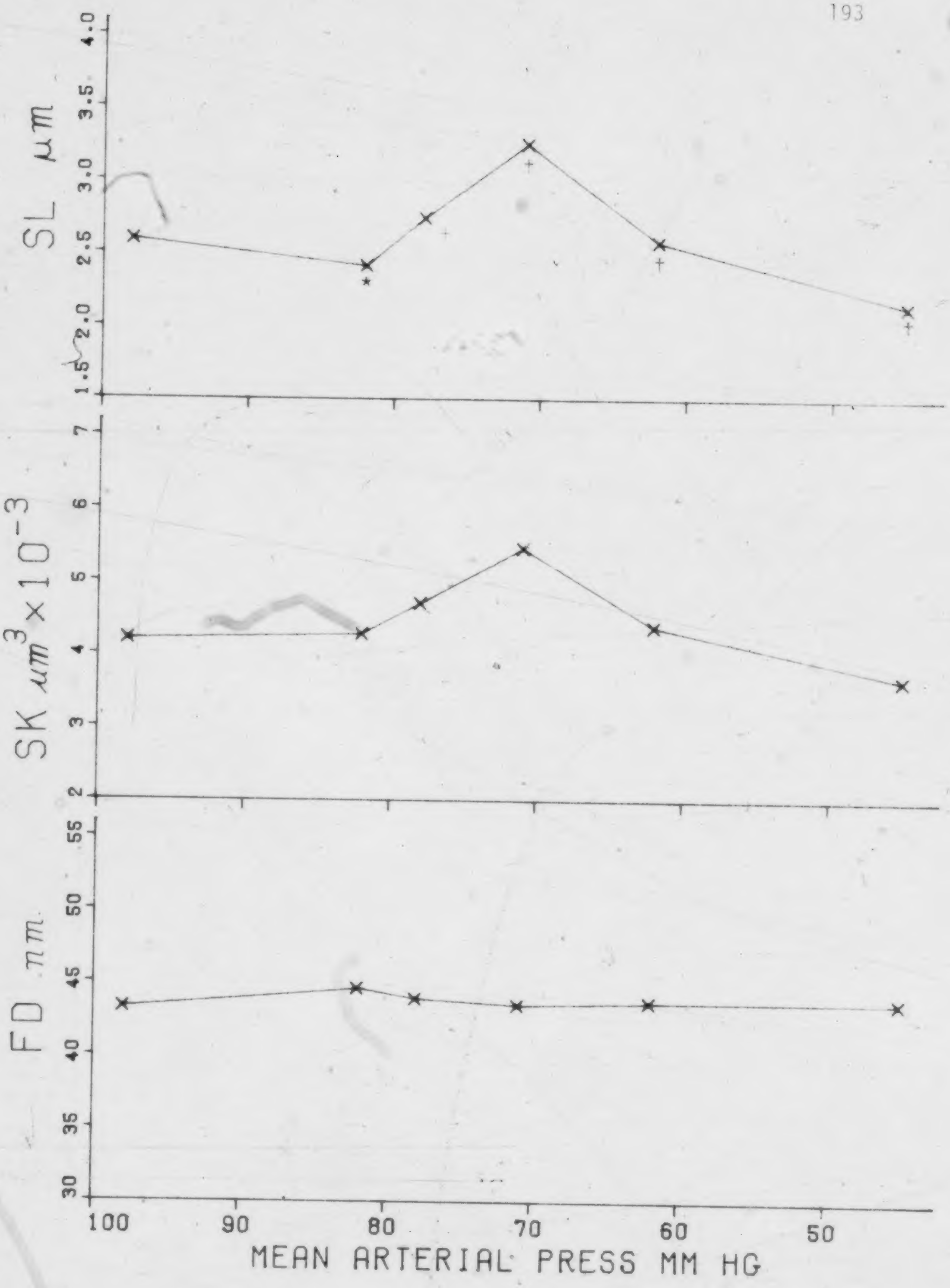

SL μm
1.5
2.0
2.5
3.0
3.5
4.0
SK μm³×10⁻³
2
3
4
5
6
7
FD nm
30
35
40
45
50
55
100
90
80
70
60
50
MEAN ARTERIAL PRESS MM HG

Figure 27

Series V - Adductor muscles; 2.5% glutaraldehyde immersion fixation. Cell volume (FD) does not, until 70 mm Hg, change. Below this arterial pressure there is a significant reduction in IFD ($P<0.01$). However only the final sarcomere length (SL) is significantly increased ($P<0.01$) and consequently unit cell volume (SK) is also increased at 45 mm Hg arterial pressure. This implies interference with contraction at lower arterial pressures. Since capillary pressure (Figure 15) is normal, reduced flow is probably present.

(§$P<0.001$; †$P<0.05$; ¶$P<0.01$)

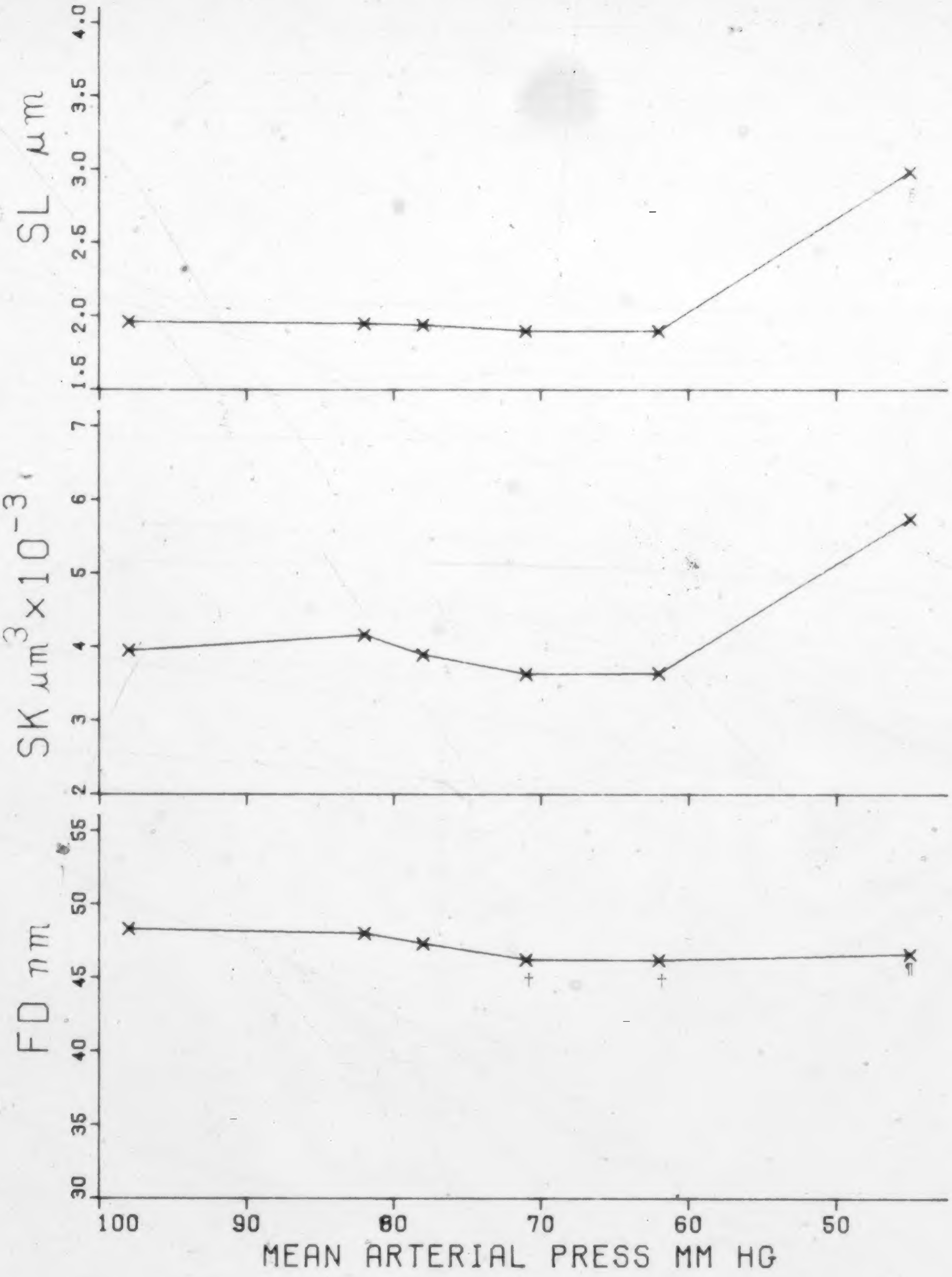

SL μm
1.5
2.0
2.5
3.0
3.5
4.0
SK μm³×10⁻³
2
3
4
5
6
7
FD nm
30
35
40
45
50
55
†
†
¶
100
90
80
70
60
50
MEAN ARTERIAL PRESS MM HG

Figure 28

Series VI - Adductor brevis; 2.5% glutaraldehyde immersion fixation. The plotted values represent the average of the values obtained from 2 rats. These values were grouped after t-test comparisons showed no difference between the two animals at each pressure. Cell volume does not change until 30 mm Hg when it is increased ($P<0.01$). Sarcomere length is also significantly increased ($P<0.01$).

(¶$P<0.001$; †$P<0.01$; *$P<0.05$)

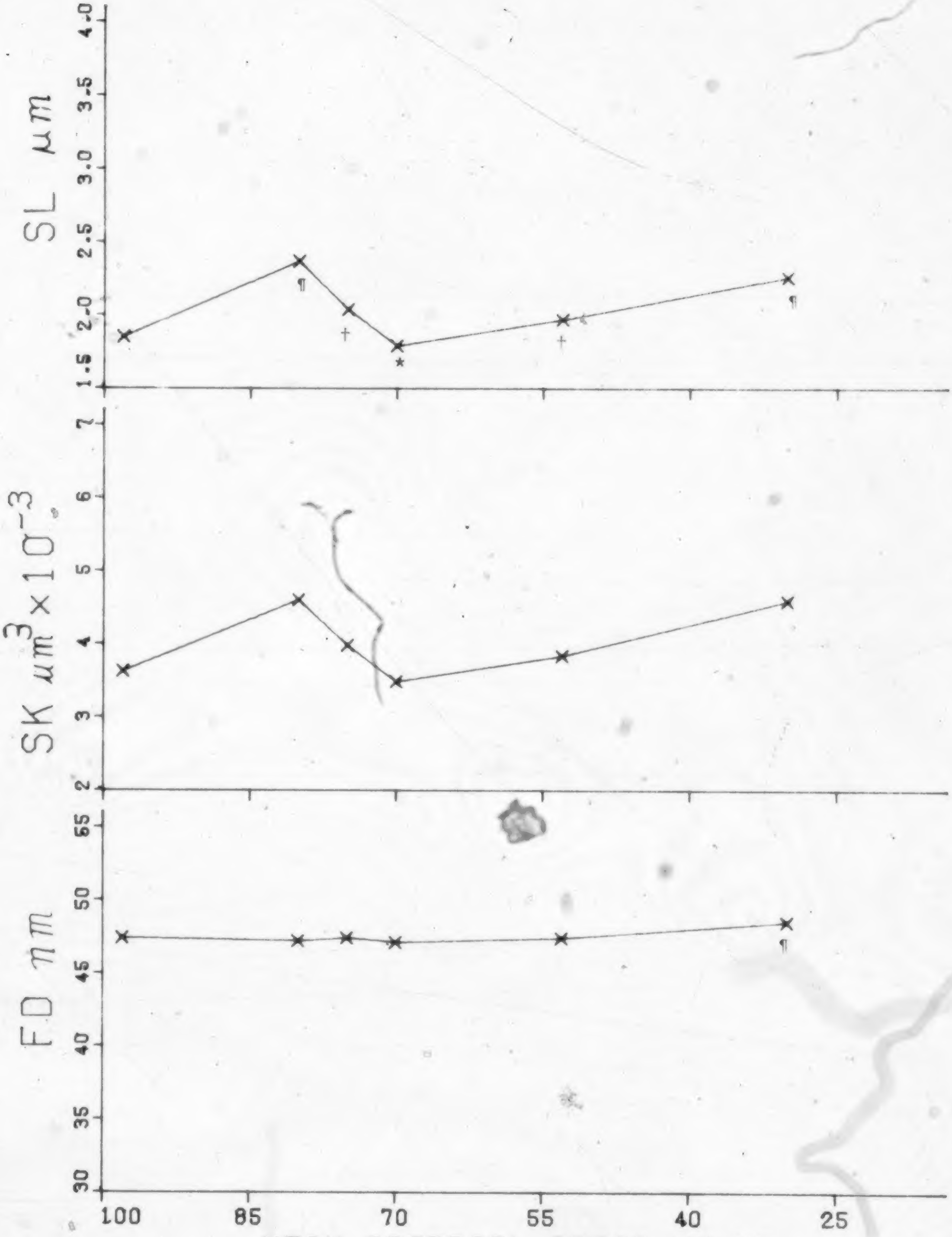
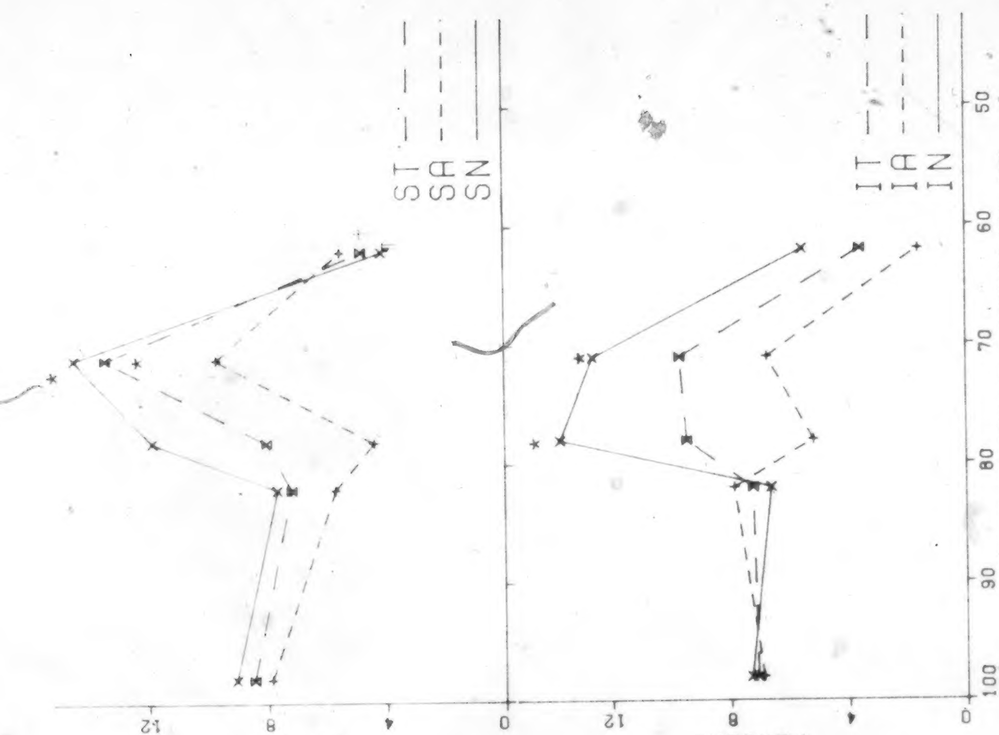

SL µm
1.5
2.0
2.5
3.0
3.5
4.0
¶
†
*
†
¶
SK µm3×10-3
2
3
4
5
6
7
FD nm
30
35
40
45
50
55
¶
100
85
70
55
40
25
MEAN ARTERIAL PRESS MM HG

Figure 29

Series I (Lumbricals fixed by arterial perfusion of 1% glutaraldehyde). Volume fraction of mitochondria during shock. As the arterial pressure diminishes, the volume of mitochondria changes considerably. The volume increases slightly in the center of the cell (AA) ($P<0.10$) but mitochondria virtually disappear from the edge of the cell (AN) ($P<0.01$). I-band mitochondria also change significantly. This may represent a shift of mitchondria from the A-band to the I-band during shock. Cross-correlation of figures 23 and 29 show that total mitochondrial volume change bears no relationship to changes in cell volume ($r=-0.48$; $P>0.10$). Similarly A-band VM and I-band VM also show no correlation with cell volume ($r= -0.30$; $P>0.10$; $r= -0.36$, $P>0.10$ respectively).

(*$P<0.10$; †$P<0.05$; ¶$P<0.01$)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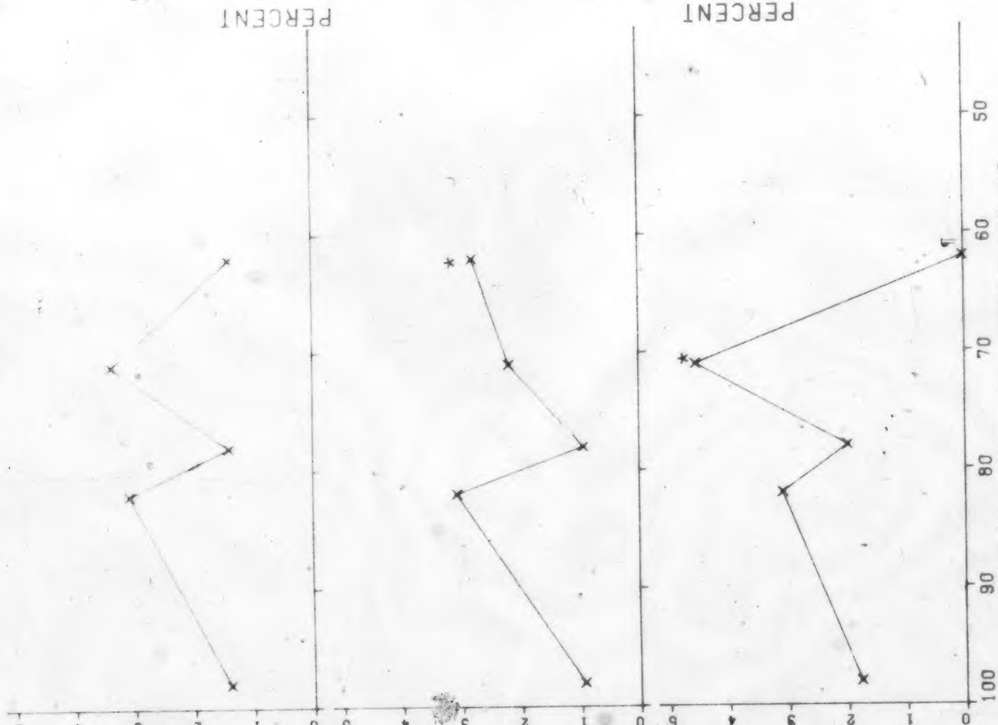
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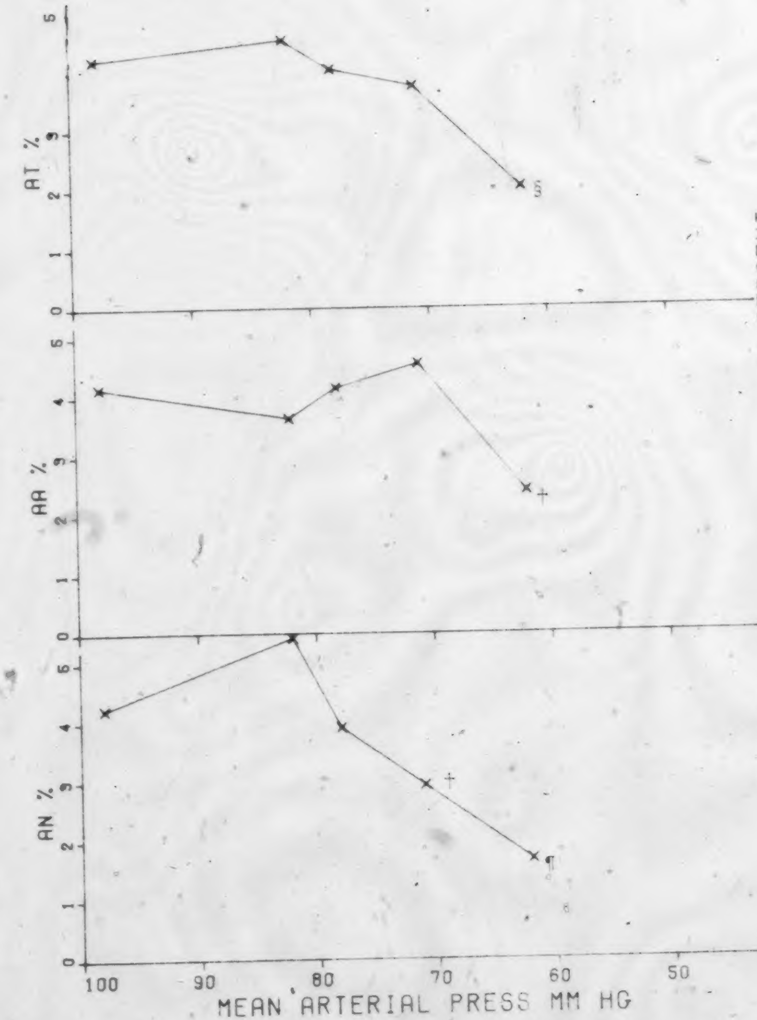
Figure 30

Series I – Sarcoplasmic reticulum volume. SR volume is significantly decreased in all areas of the cell at 60 mm Hg. Total and I-band SR appears to follow a similar pattern of change as mitochondrial volume. However, cross-correlation of Fig. 30 with Fig. 29 shows a highly significant negative relationship between total SR volume and mitochondrial volume ($r = -0.94$, $P < 0.01$), and I-band volumes ($r = -0.84$, $P < 0.05$). A slight positive relationship between A-band SR volume and A-band mitochondria volume is also present ($r = 0.72$, $P < 0.10$). This is expected due to the functional relationship between the two organelles described in the text.

(§$P < 0.001$; ¶$P < 0.01$, †$P < 0.05$)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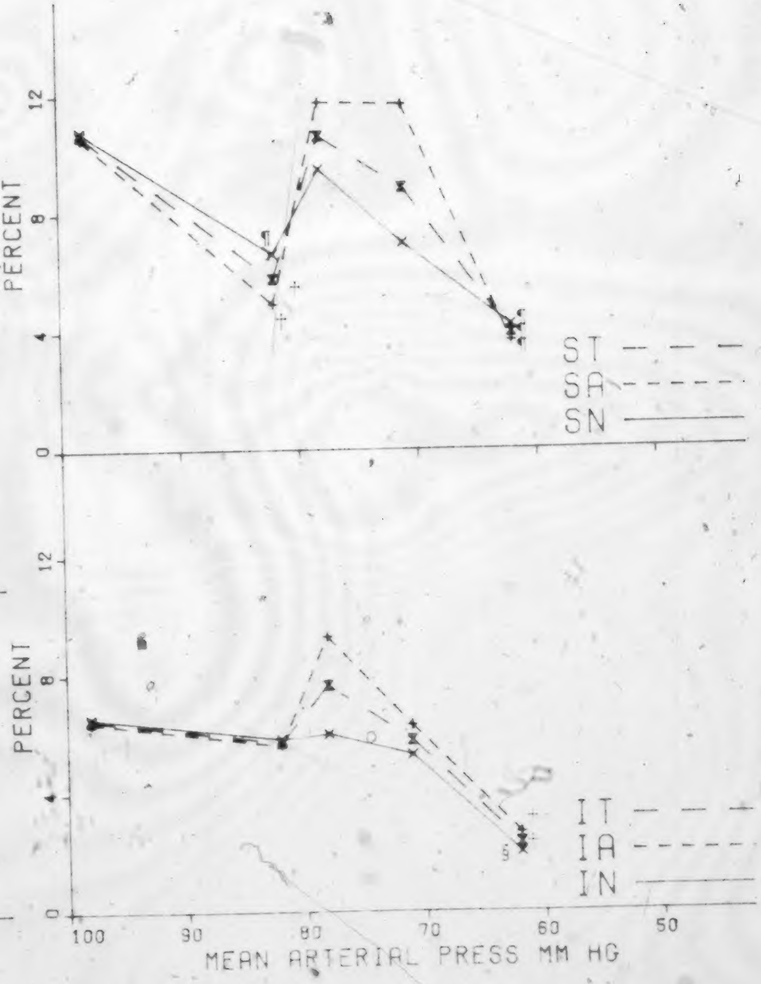
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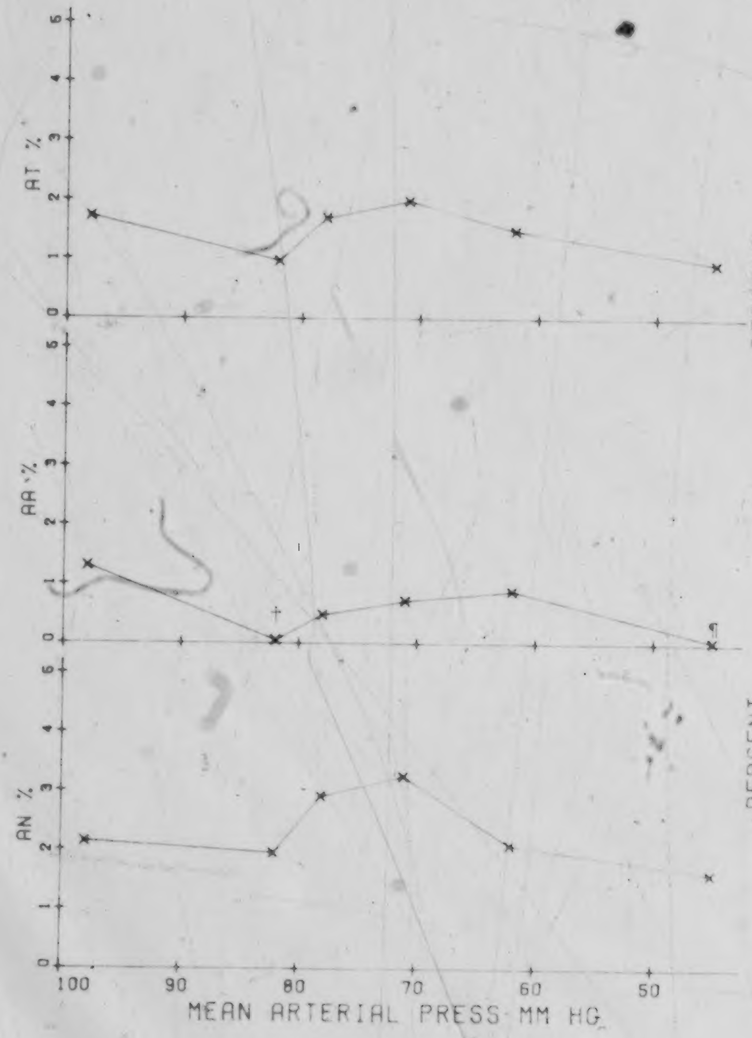
Figure 31

Series II - (Lumbricals fixed by arterial perfusion of 2.5% glutaraldehyde). Mitochondrial volume. Mitochondria in AA almost disappear with marked decreases in capillary pressure. I-band and total mitochondrial volume vary slightly. Significant inverse relationship to SR volume (Fig. 32) is seen in the I-band volume (IT) ($r= -0.97$, $P<0.01$) and total volume ($r= -0.94$, $P<0.01$). This probably reflects a (ST) translocation of water from one organelle to the other during shock. The slightly positive relationship seen in Series I for A-band SR and mitochondria volumes is not evident ($r= 0.70$, $P>0.10$). There is no relationship between mitochondria and cell volume (Fig. 24) in the A-band ($r= 0.13$, $P>0.10$), I-band ($r= 0.12$, $P>0.10$) or total volumes ($r= 0.49$, $P>0.10$). Consequently, changes in mitochondrial volume are not representative of changes in either cell volume or sarcoplasmic reticulum volume.

(*$P<0.10$; †$P<0.05$; ¶$P<0.01$)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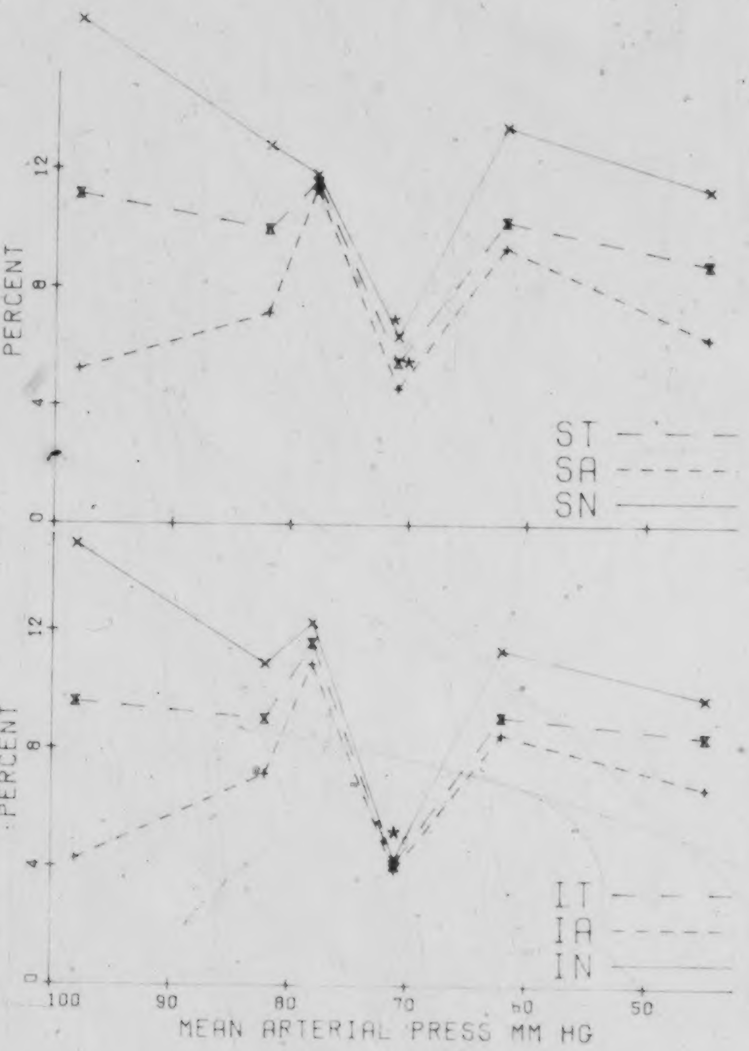
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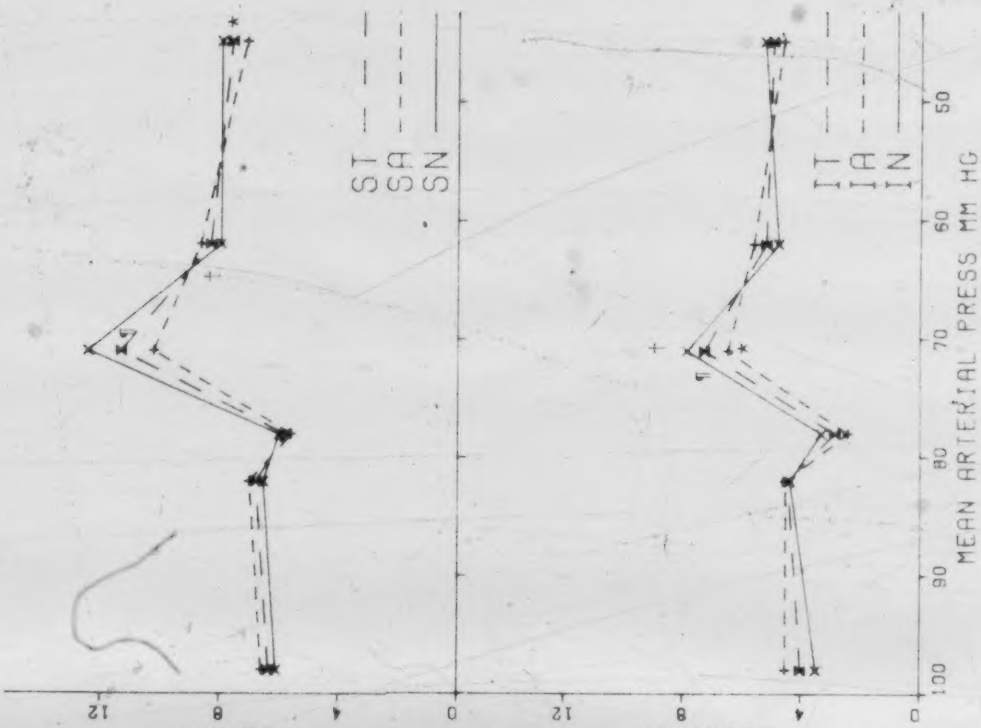
Figure 32

Series II - Sarcoplasmic reticulum volume during shock. Changes in SR volume and mitochondrial volume (Fig. 31) are also consistent with the known relationships of mitochondria and SR during contraction. Changes in SR volume have no correlation with cell volume (Fig. 24) in the A-band ($r= -0.14$, $P>0.10$), I-band ($r= -0.33$, $P>0.10$) or total cell ($r= -0.31$, $P>0.10$). This is also true for SR volume and sarcomere length ($r= -0.27$, $P<0.10$). Consequently SR volume changes during shock bear no relationship to cell volume or state of contraction. Consequent cell osmotic activity and SR volumes are also unrelated.

($^*P<0.01$; $^†P<0.05$; $^¶P<0.01$)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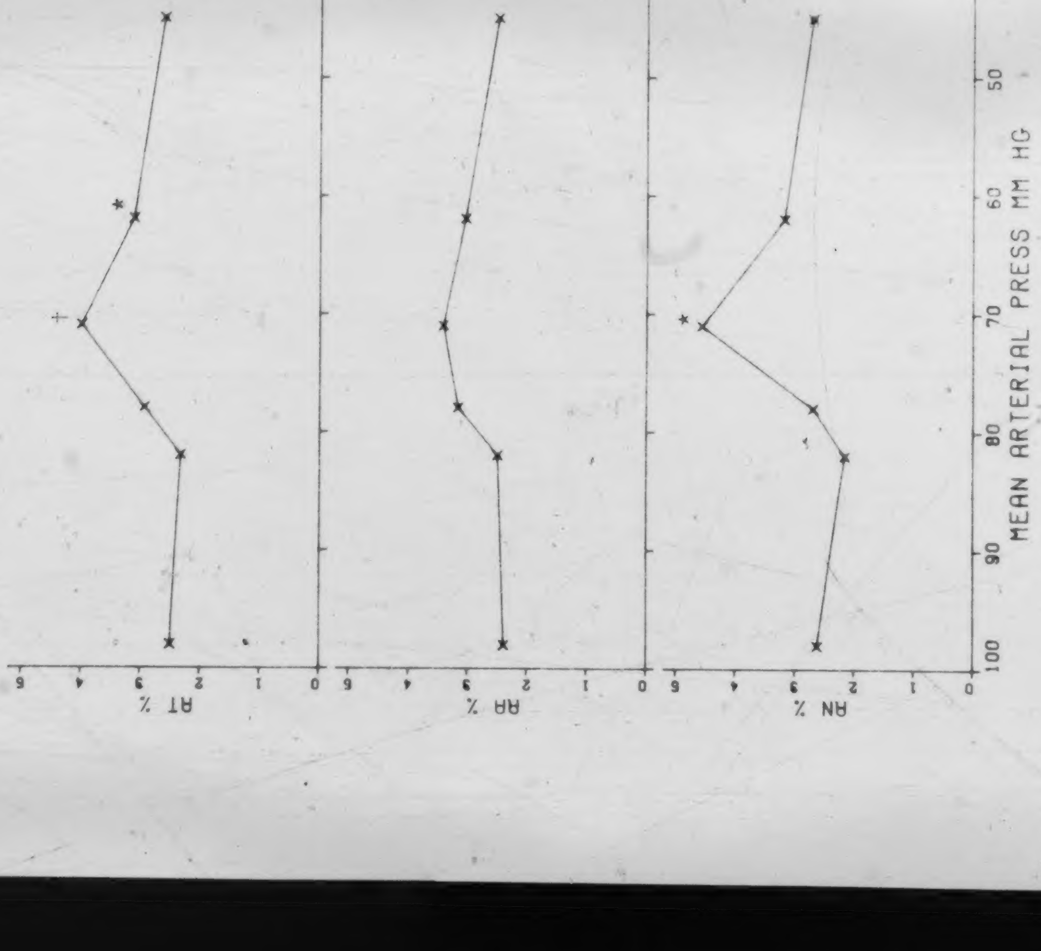
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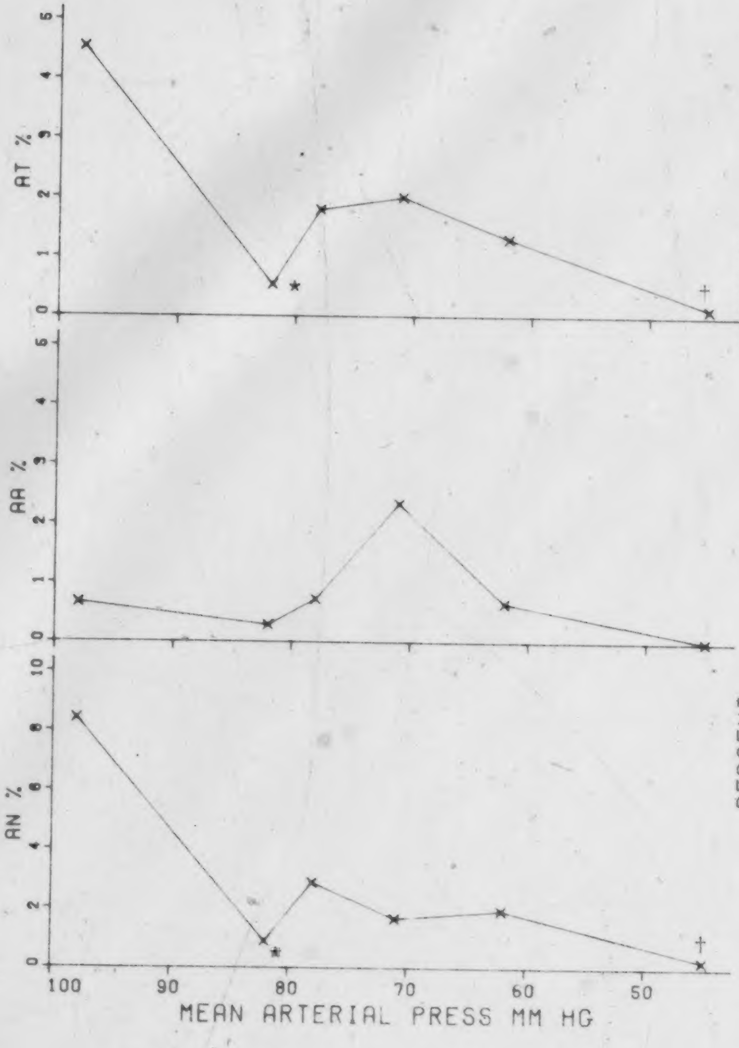
Figure 33

Series III - (Lumbricals fixed by immersion in 2.5% glutaraldehyde). Mitochondria volumes. Note the virtual absence of mitochondria in the A-band at the lowest arterial and capillary pressures. Changes in I-band and total mitochondrial volume are not as severe as in Series II. However, there is again an inverse relationship between I-band mitochondrial volume and SR volume (Fig. 34), ($r = -0.87$, $P<0.05$) and independence of VM from cell volume (Fig. 25) ($r = 0.60$, $P>0.10$), and sarcomere length ($r = 0.49$, $P>0.10$). Consequently mitochondrial volume does not relate to cell volume as cell osmotic activity is reflected by unit cell volume. There is a very significant positive relationship between mitochondrial volume change during shock and extracellular fluid volume change ($r = 0.92$, $P<0.01$).

(*$P<0.10$, †$P<0.05$)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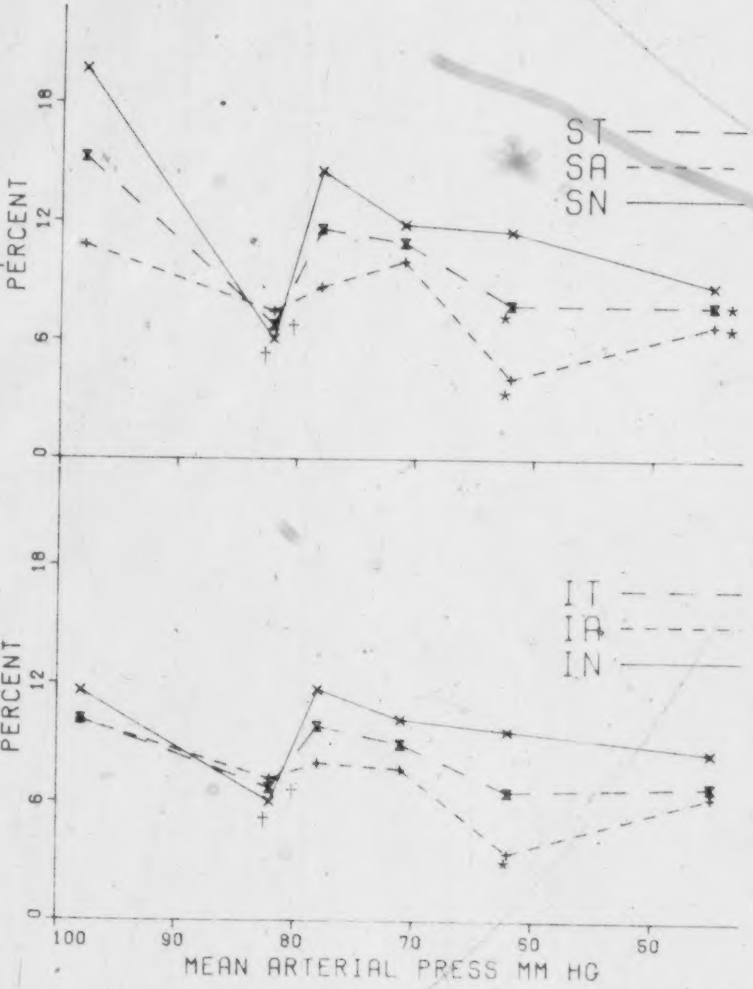
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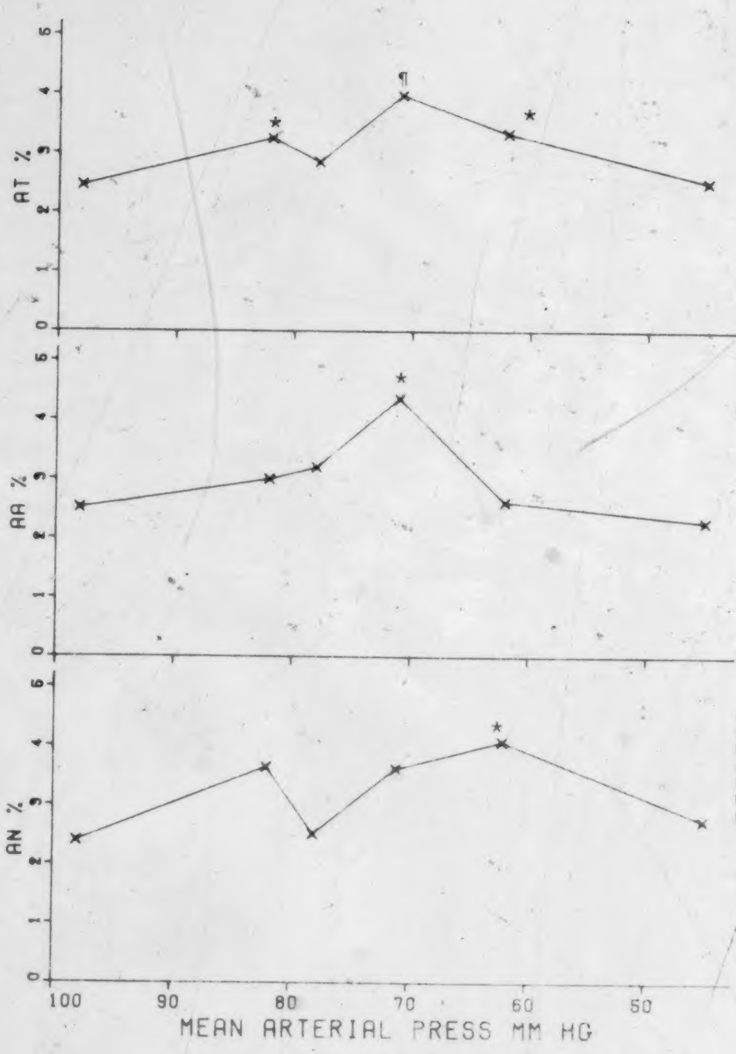
MEAN ARTERIAL PRESS MM HG

Figure 34

Series III - SR volume. The changes in SR volume are inversely related to cell volume (Fig. 25) ($r = -0.84$, $P<0.05$) and LS ($r = -0.82$, $P<0.05$). SR volume is slightly increased ($P<0.10$) at the end of the early stage of shock. On the other hand, VM has slightly decreased ($P<0.10$) at the end of early shock. This may reflect a transfer of fluid between the two organelles.

(*$P<0.10$; †$P<0.05$; ¶$P<0.01$)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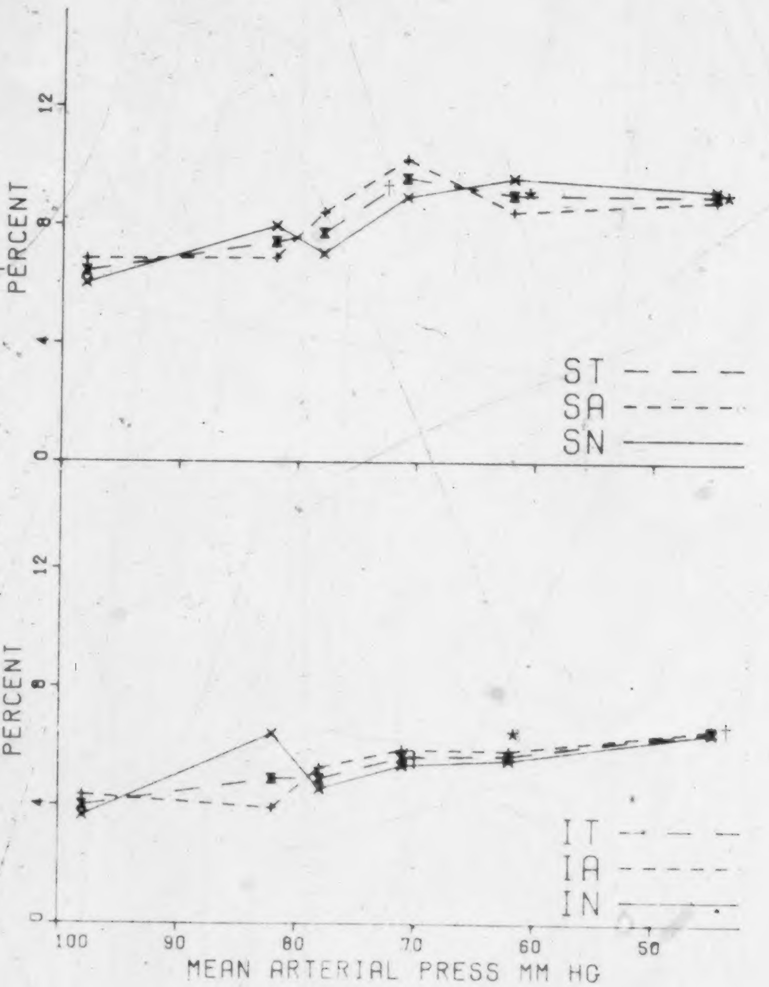
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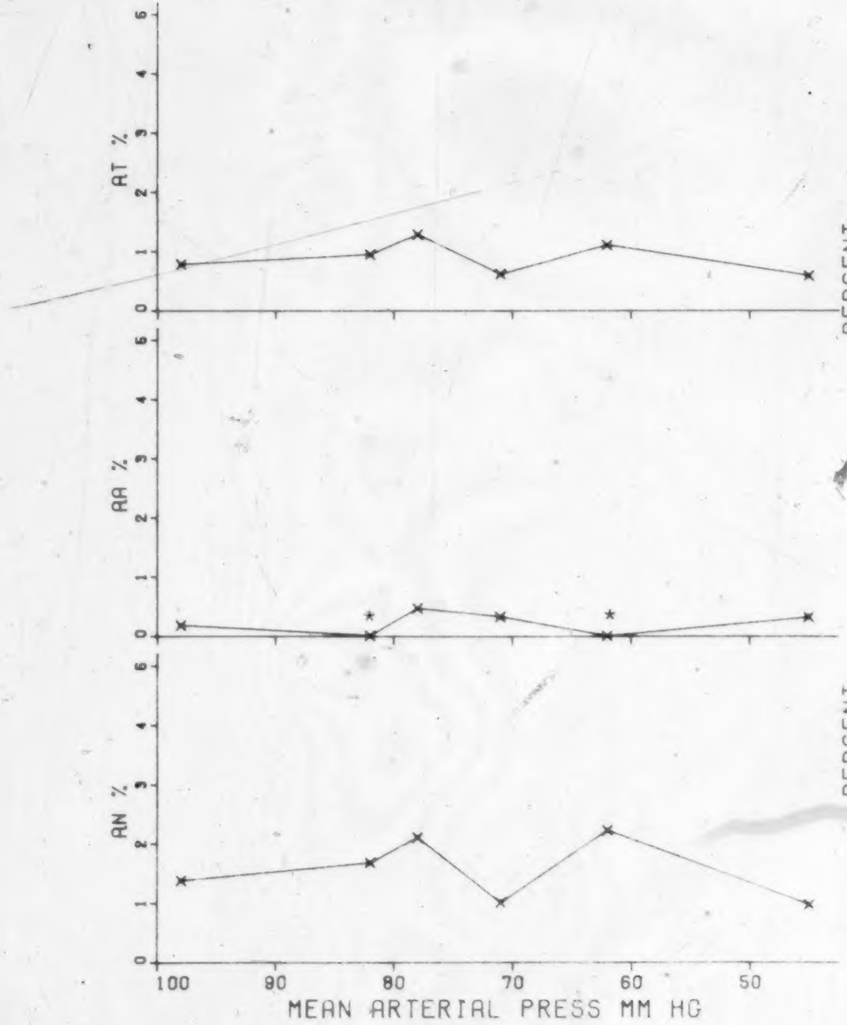
Figure 35

Series IV - (Adductor brevis fixed by arterial perfusion of 2.5% glutaraldehyde). Mitochondria volume. There is minimal decrease change in A-band volume in the center of the cell (AA) but a significant increase in I-band and total volume as the arterial pressure decreases below 80 mm Hg. No correlation with cell volume exists ($r= 0.13$, $P>0.10$) but a highly significant positive correlation with extracellular space volume (Fig. 44) during shock does exist ($r= 0.91$, $P<0.01$).

(*$P<0.10$; †$P<0.05$; ¶$P<0.01$)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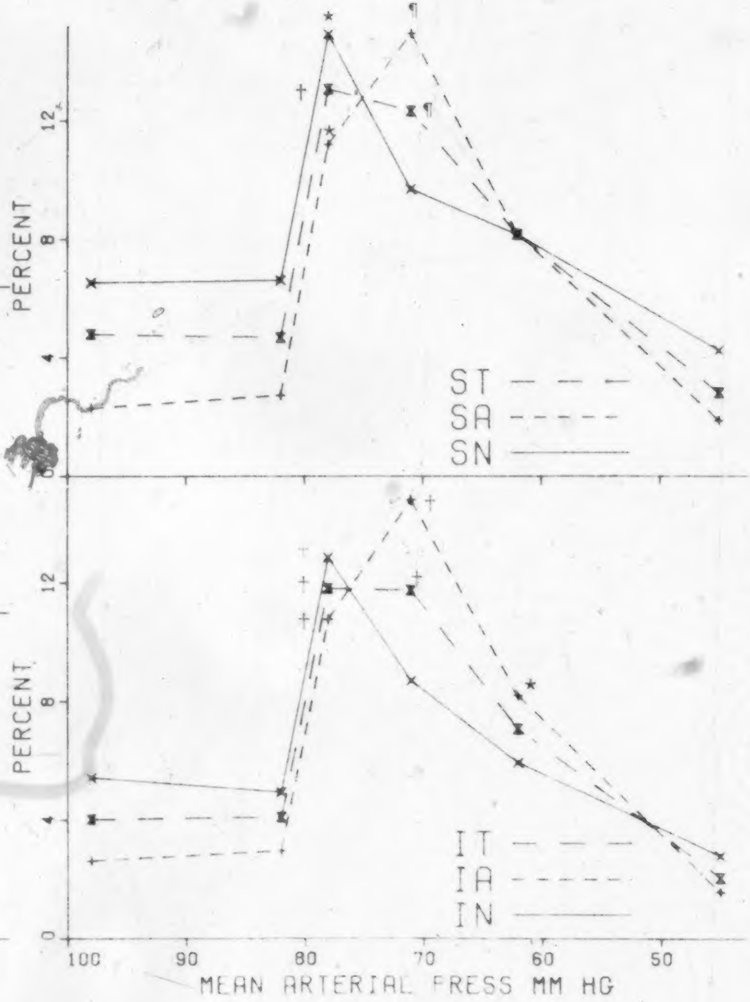
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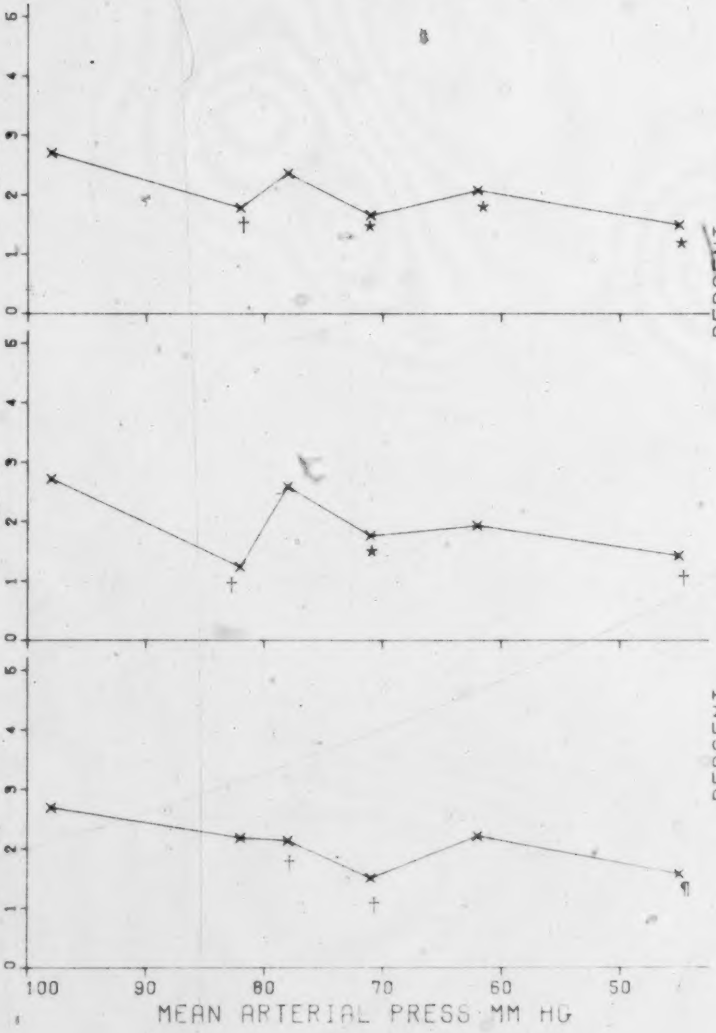
MEAN ARTERIAL PRESS MM HG

Figure 36

Series IV - Sarcoplasmic reticulum volume. There is a persistant and significant decrease in all components of SR. Total SR volume at 45 mm Hg arterial pressure is significantly decreased ($P<0.001$). There is no relationship between SR volume and cell volume (Fig. 26) ($r= -0.55$, $P>0.10$) or SR volume and sarcomere length ($r= 0.02$, $P>0.10$). This is opposite to the known relationship between SR and the state of contraction.

(*$P<0.10$; +$P<0.05$; ¶$P<0.01$)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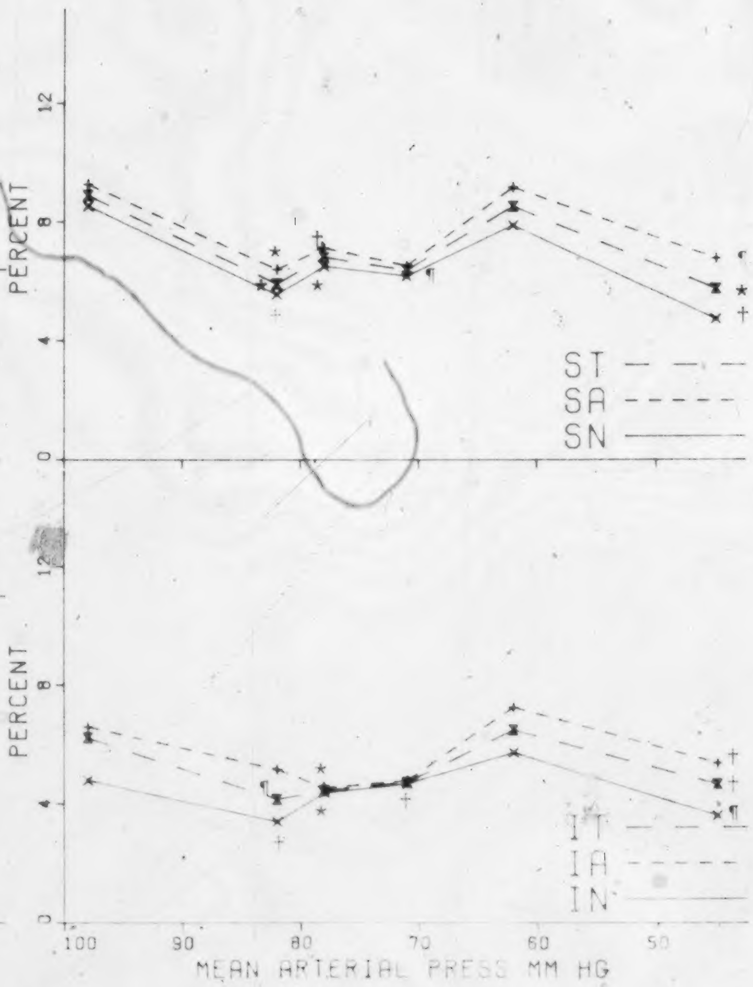
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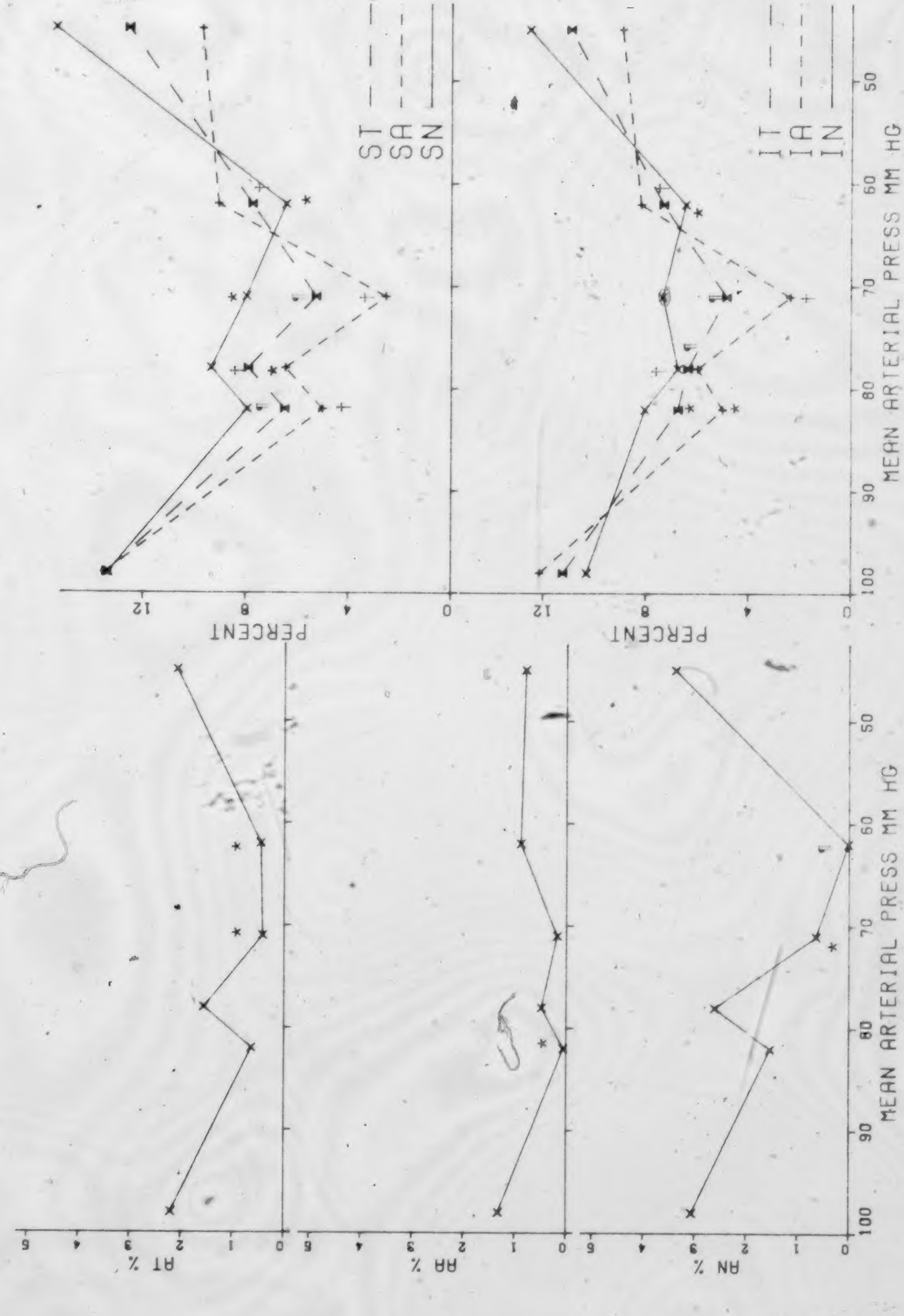
MEAN ARTERIAL PRESS MM HG

Figure 37

Series V - (Adductor brevis fixed by immersion in 2.5% glutaraldehyde). Mitochondria volume. A shift of mitochondria from the A-band is seen as arterial pressure decreases. I-band and total SR volume follow a similar pattern. There is no correlation with cell volume (Fig. 27) ($r = 0.36$, $P > 0.10$) or sarcomere length ($r = 0.48$, $P > 0.10$).

(*$P < 0.10$; †$P < 0.05$; ¶$P < 0.01$)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MEAN ARTERIAL PRESS MM HG
AT %
AA %
AN %
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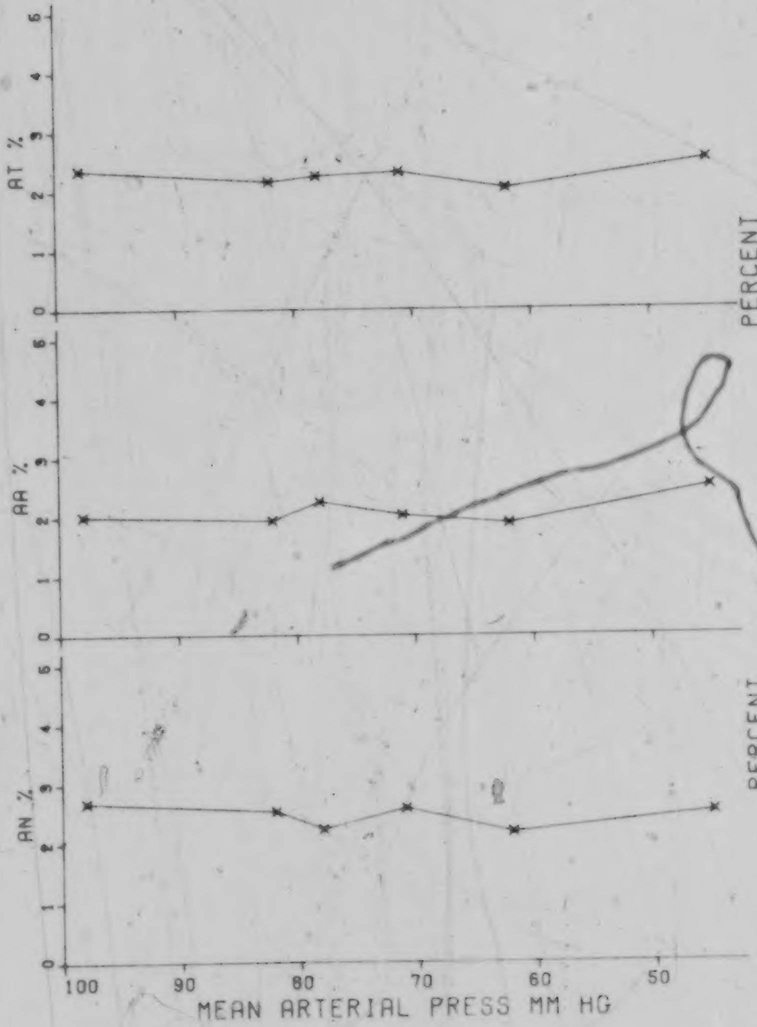
3
2
1
0

Figure 38

Series V - Sarcoplasmic reticulum volume. A-band SR does not change significantly in contrast to series IV. A similar finding was present for series II and III, perhaps indicating a different effect on tissue by the two methods of fixation. Total SR volume (ST) decreases slightly at 45 mm Hg ($P<0.10$) I-band SR also decreases ($P<0.05$).

(*$P<0.10$; †$P<0.05$)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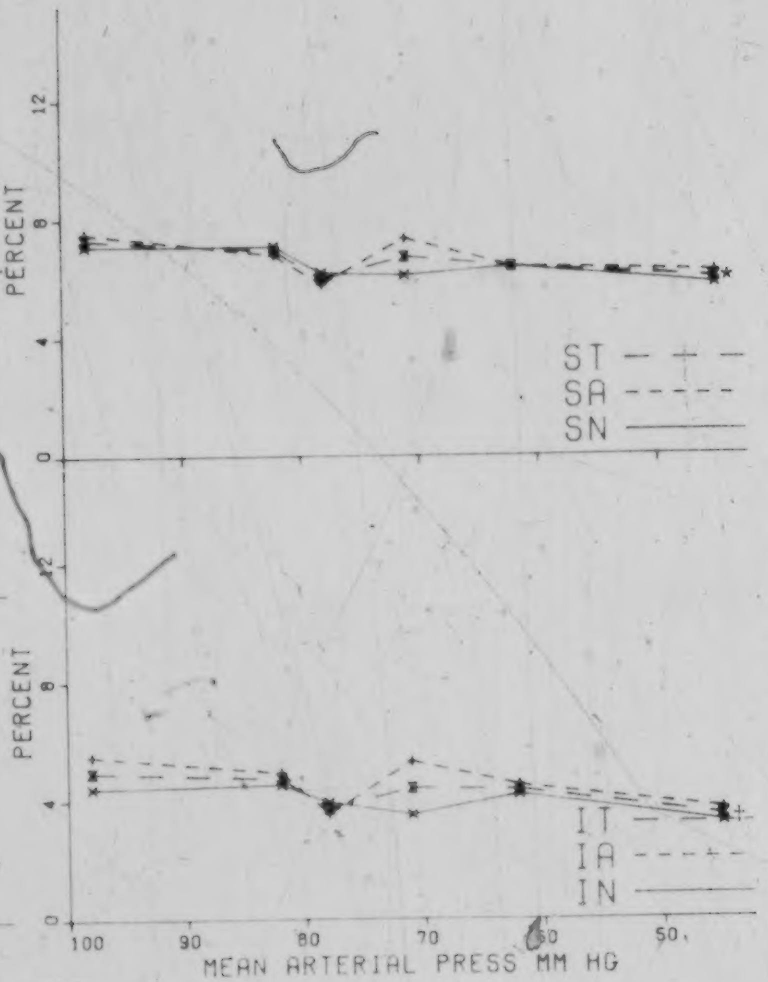
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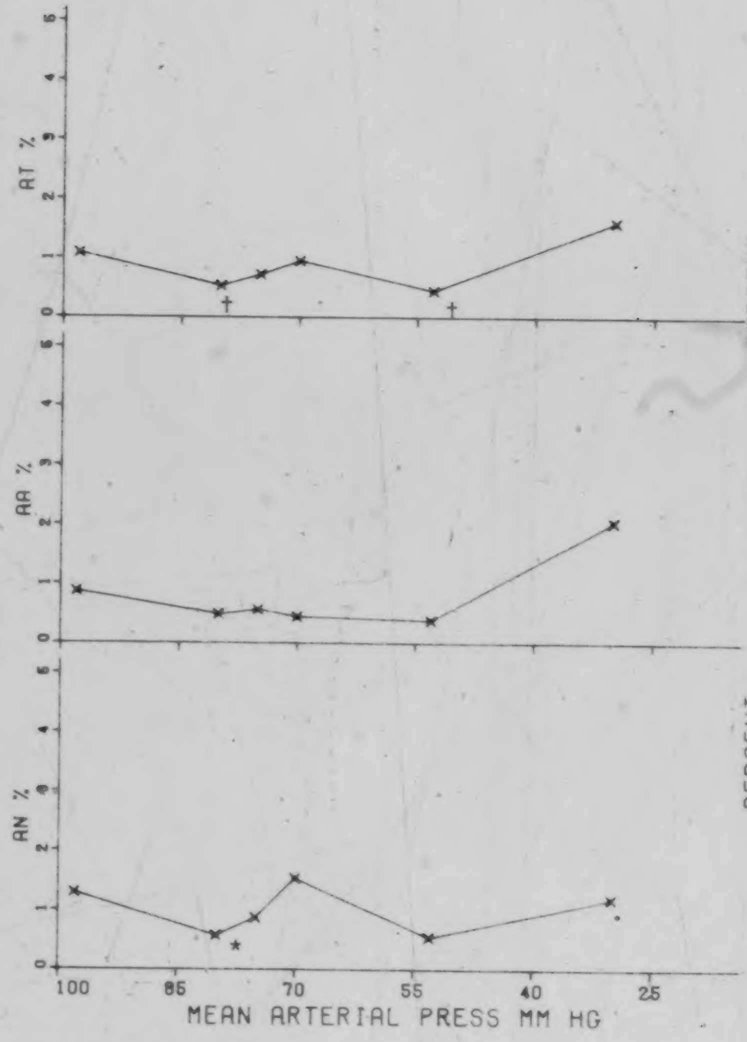
MEAN ARTERIAL PRESS MM HG

Figure 39

Series VI - (Adductors fixed by immersion in 2.5% glutaraldehyde). Mitochondria volume. VM is not related to cell volume (Fig. 28) ($r = 0.27$, $P>0.10$) confirming similar relationships in all other series.

(*$P<0.10$; †$P<0.05$; ¶$P<0.01$)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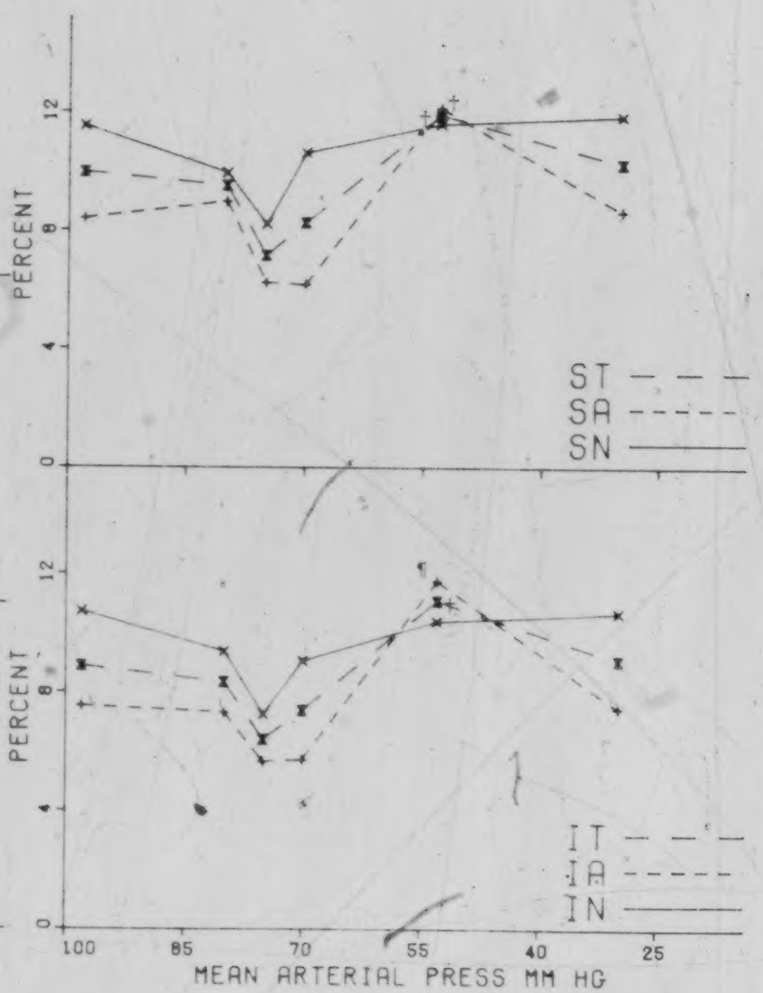
MEAN ARTERIAL PRESS MM HG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70
55
40
25
MEAN ARTERIAL PRESS MM HG

Figure 40

Series VI - Sarcoplasmic reticulum volume. SR volume in the A-band does not change significantly. However, total SR volume increases slightly ($P<0.10$). SR volume shows marginal correlation with interstitial space volume (Fig. 45) ($r= 0.72$, $P<0.10$). Does this represent the communication suggested by Birks (1965)?

($^*P<0.10$; $^+P<0.05$)

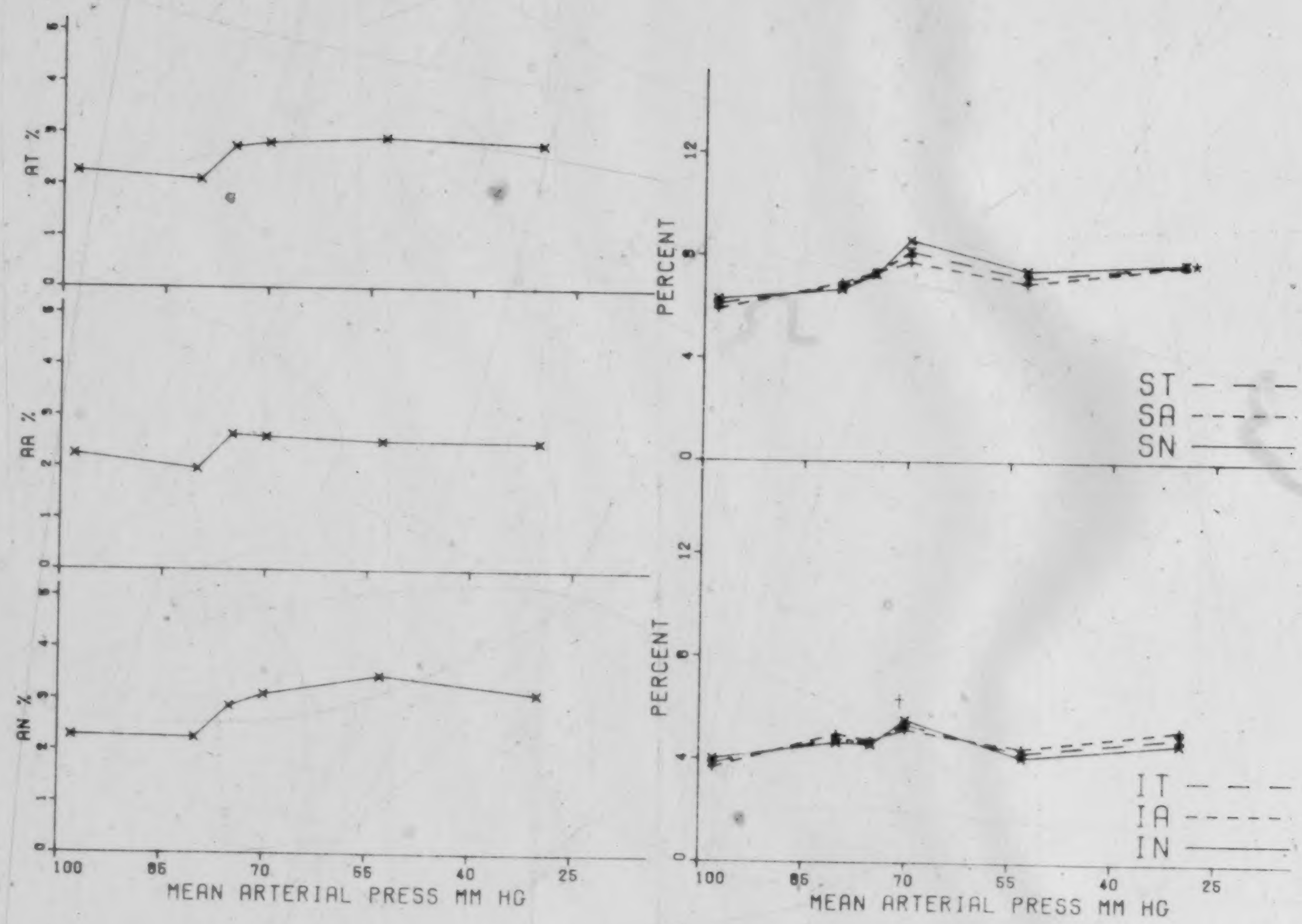
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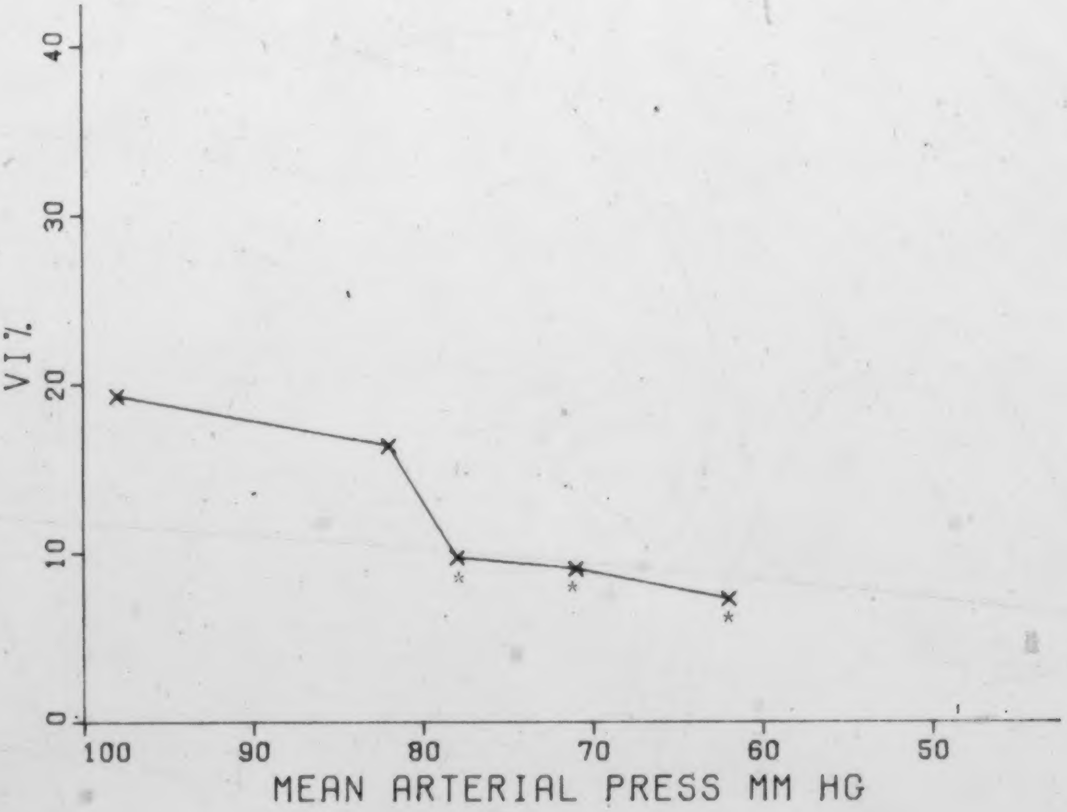
Figure 41

Series I - Interstitial space volume decreases by 62% at 60 mm Hg. This decrease in VI is related to a loss of fluid from VI (5.5%) due to a significant drop in capillary pressure.

($^*P<0.01$)


VI%
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100
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80
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MEAN ARTERIAL PRESS MM HG
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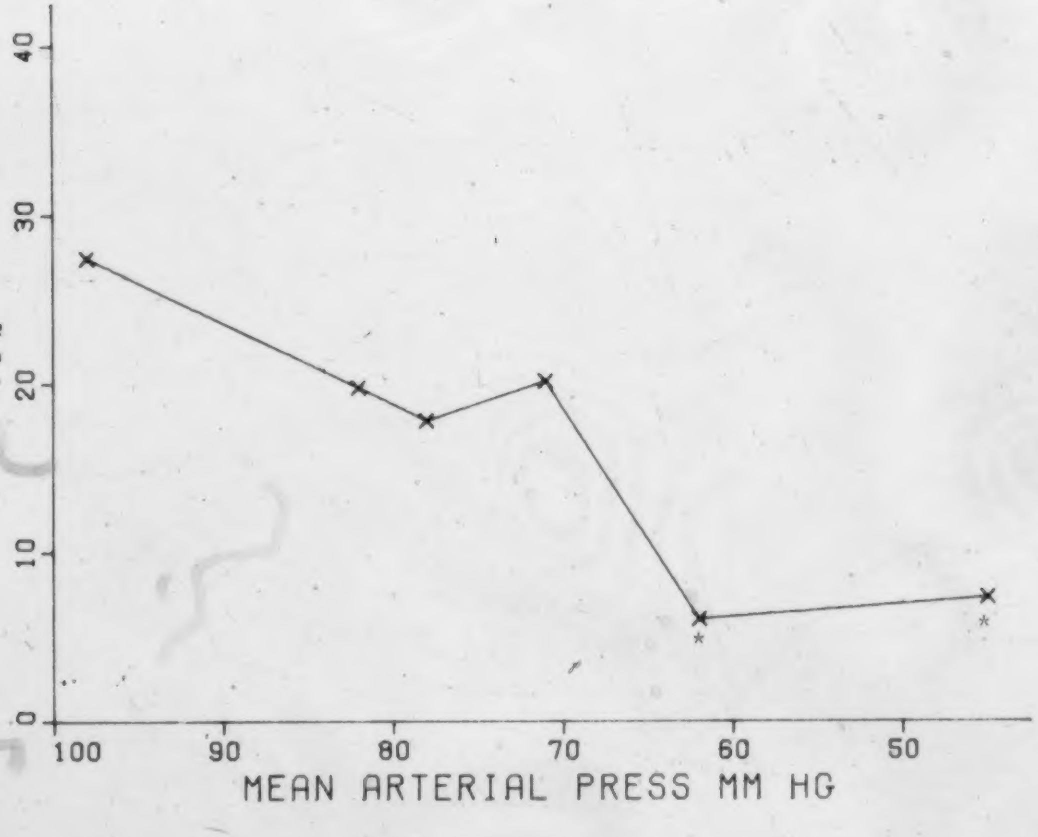
Figure 42

Series II - Interstitial space volume. VI decreases by 78% or 21% of tissue volume. Since cell volume is normal at 45 mm Hg the decrease in VI is due to a loss of water from the interstitial space into the vascular system, and not due to cell swelling.

(*$P<0.05$)


40
30
20
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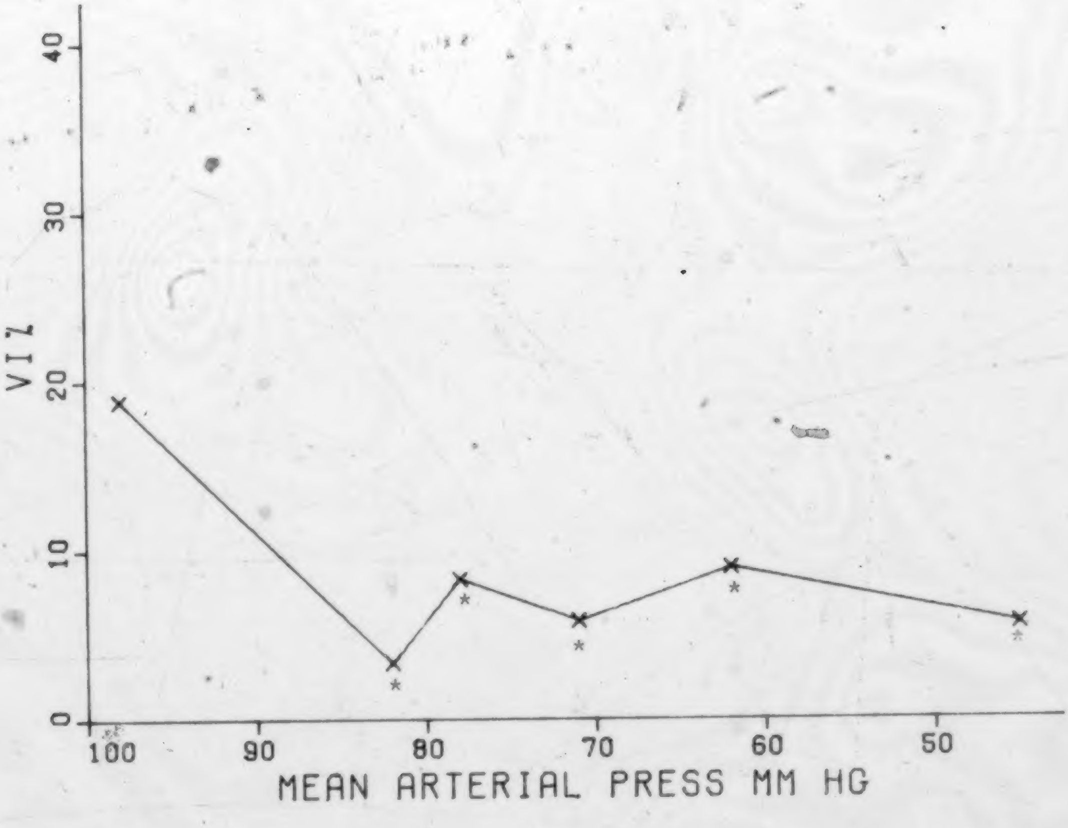
0
VI%
100
90
80
70
60
50
MEAN ARTERIAL PRESS MM HG
*
*

Figure 43

Series III - Interstitial space volume decreases by 70 ($P<0.01$) which is similar to the change in a muscle fixed by arterial perfusion (Fig. 42).

(*$P<0.01$)


VII
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MEAN ARTERIAL PRESS MM HG
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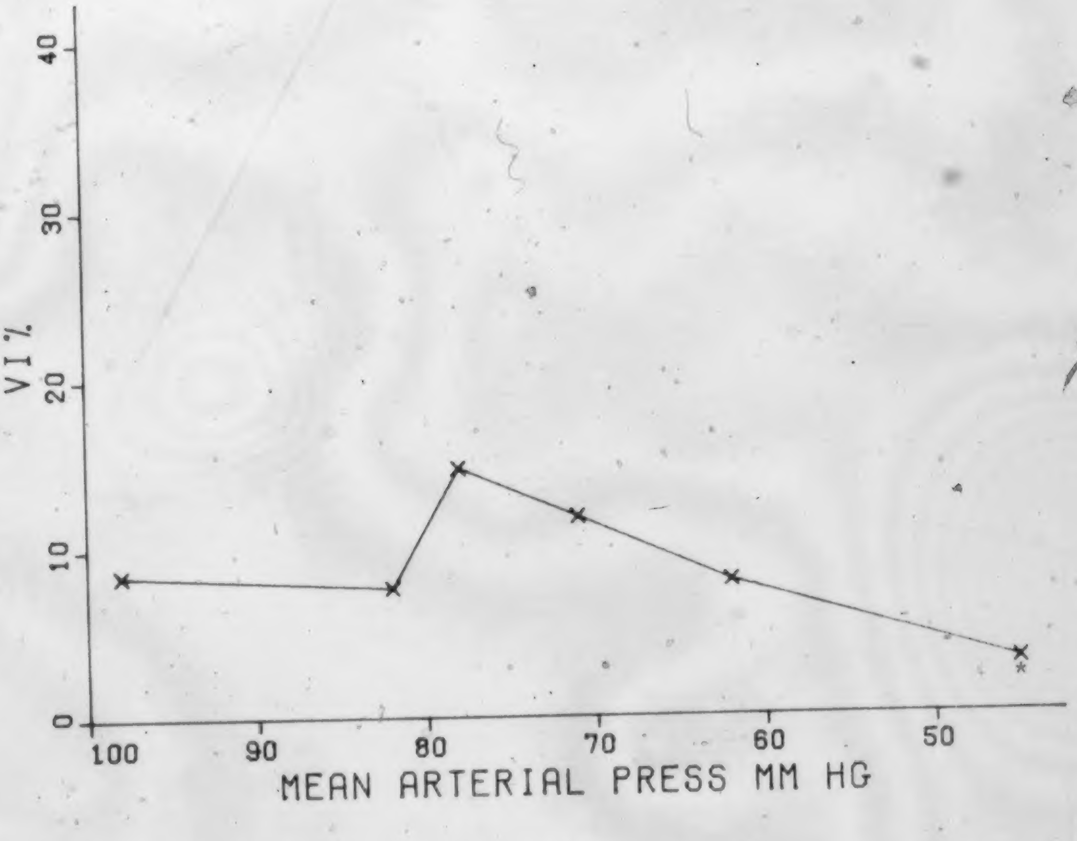
Figure 44

Series IV. Interstitial space volume decreases by 63%. (*P<0.10)


VI%
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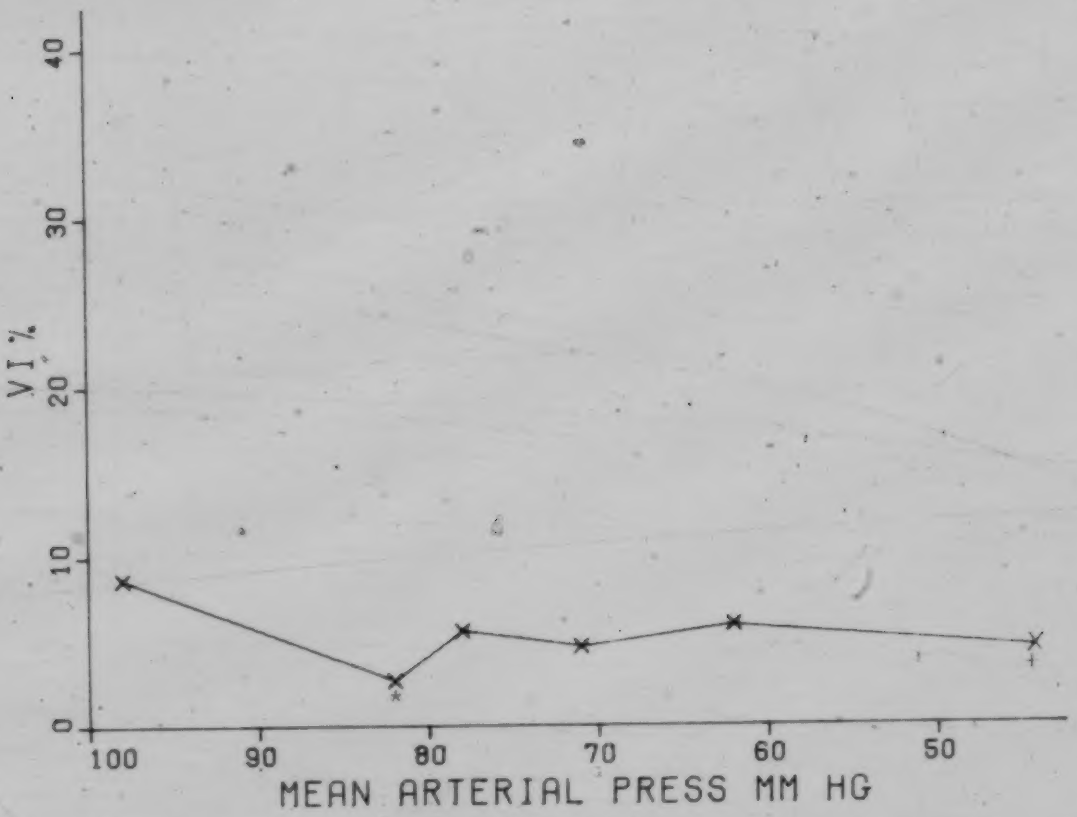
MEAN ARTERIAL PRESS MM HG

Figure 45

Series V - Interstitial space volume decreases by only 12% ($P>0.10$) in contrast to 63% in arterial perfusion fixed muscle ($P<0.10$) (Fig. 43). The only significant decrease in VI occurs with the initial decrease in arterial pressure. Is there an element of direct injury during surgical biopsy prior to fixation which accounts for this difference.

(*$P<0.05$; +$P<0.10$)


VI%
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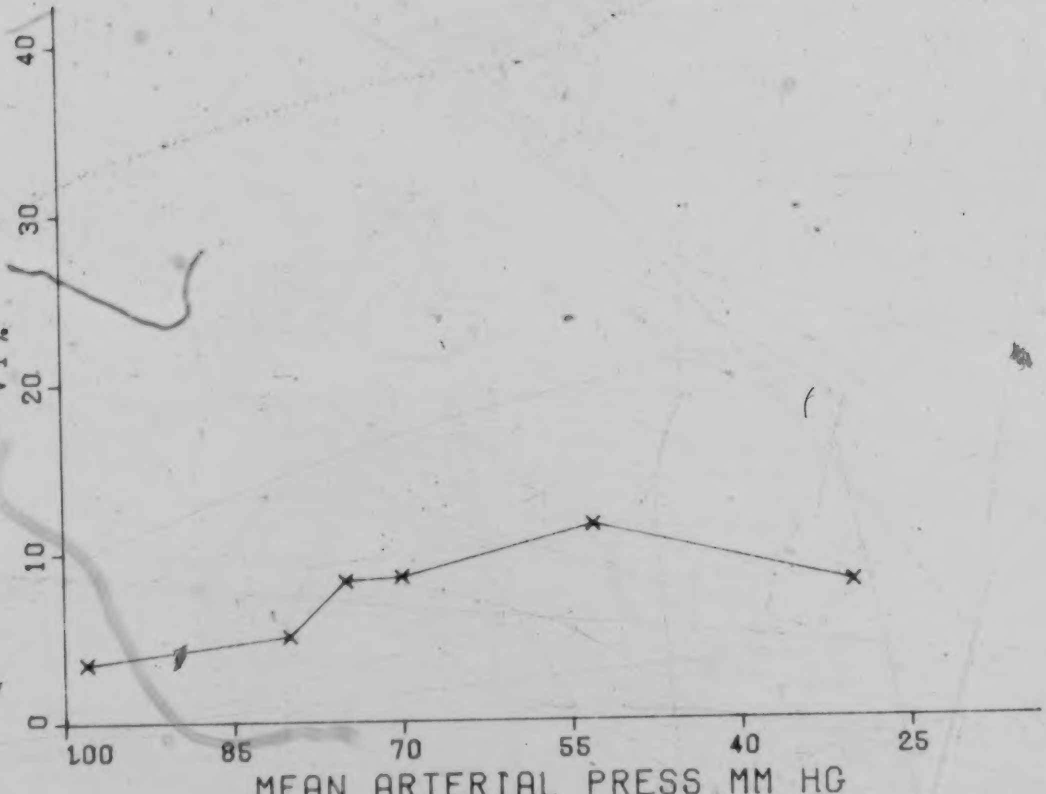
MEAN ARTERIAL PRESS MM HG

Figure 46

Series VI - Interstititial space volume appears to increase during shock in contrast to all the other series. However, none of the apparent increases are significant. Since VI decreased in all other series, VI at the lower arterial pressures has probably increased in this series. Serial biopsies may have resulted in injury to the muscle prior to fixation, thus preventing the usual decrease in VI. Hence the effect of trauma prior to fixation may preclude the use of serial biopsies for accurate extracellular fluid analysis.


VI%
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MEAN ARTERIAL PRESS MM HG

PLATES

Plate 1

Longitudinal section of skeletal muscle.

M = mitochondrion
Sr = sarcoplasmic reticulum (x15,000)
1a to 1e - Transverse sections of skeletal muscle demonstrating the characteristics within each area of the sarcomere.

1a - Z-line
1b - I-band
1c - A-band
1d - M-line
1e - H-zone

(x58,400)

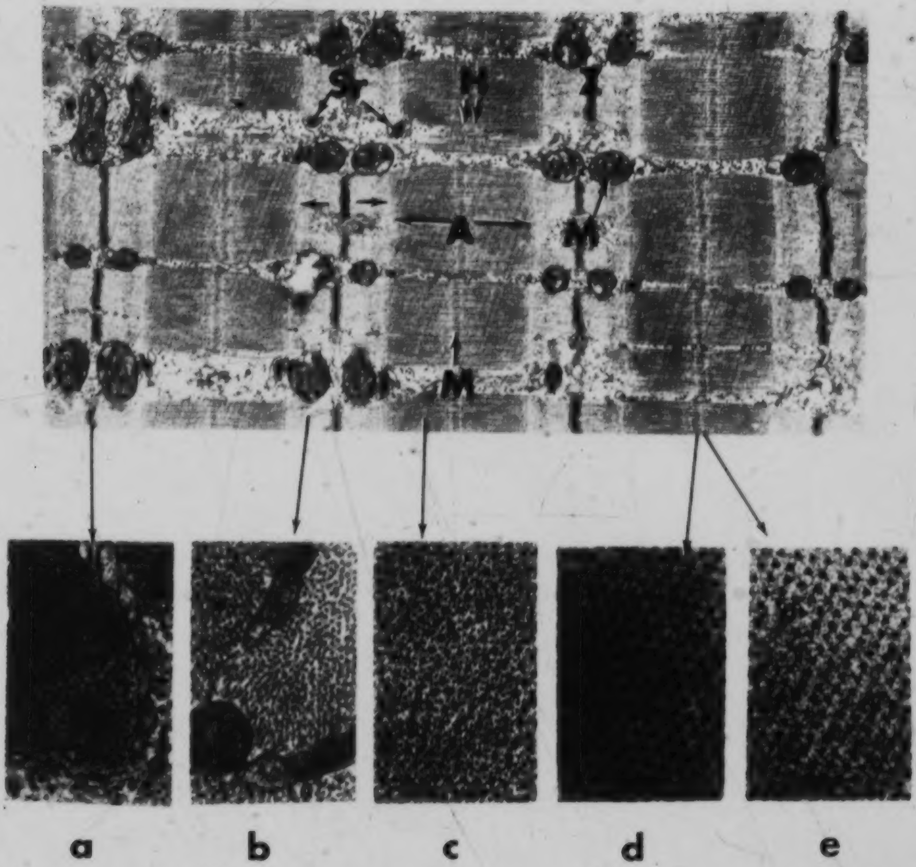

Sr
H
Z
A
M
M
a
b
c
d
e

Plate 2

Transverse section in A-band. Myofibrils (MF) are surrounded by the longitudinal components of sarcoplasmic reticulum (Sr) and by mitochondria (M) x 58,000.

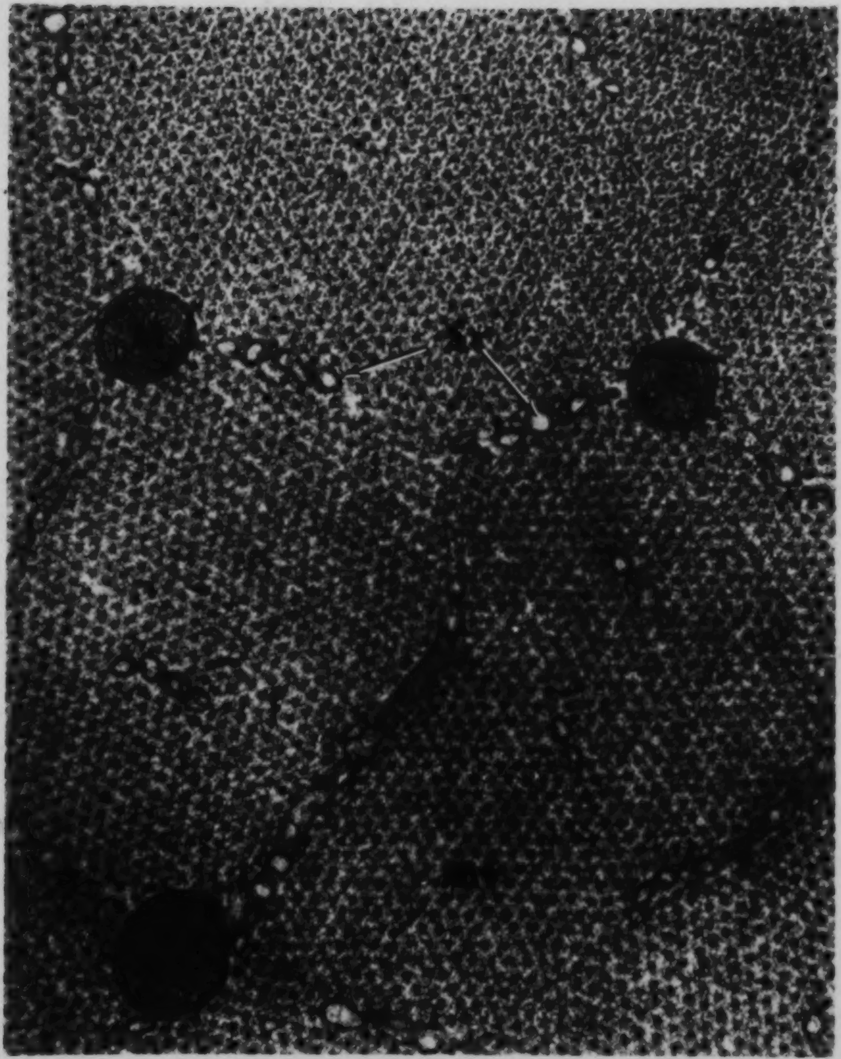
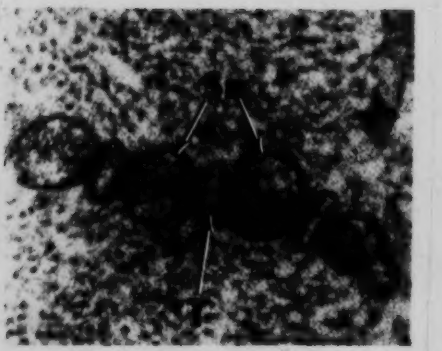


Plate 3

(a) Transverse section I-band. Note triads (central t-tubule T plus 2 Sr terminal cisternae) and bridges (short arrows) between components (x78,000).

(b) Septad. Bridges are again evident between t-tubules and terminal cisternae (x124,600).

a



b

Plate 4

Longitudinal section of triad junction. Note regularly spaced bridges (short arrows) between the t-tubule (t) and Sr (x115,500).

Sr

Plate 5

Transverse section of superficial cells of lumbrical muscle fixed by carpal tunnel perfusion of 1% glutaraldehyde. All tissues and organelles appear to be well preserved.

Z = Z-line
I = I-band
A = A-band
C = capillary

(x5,000)

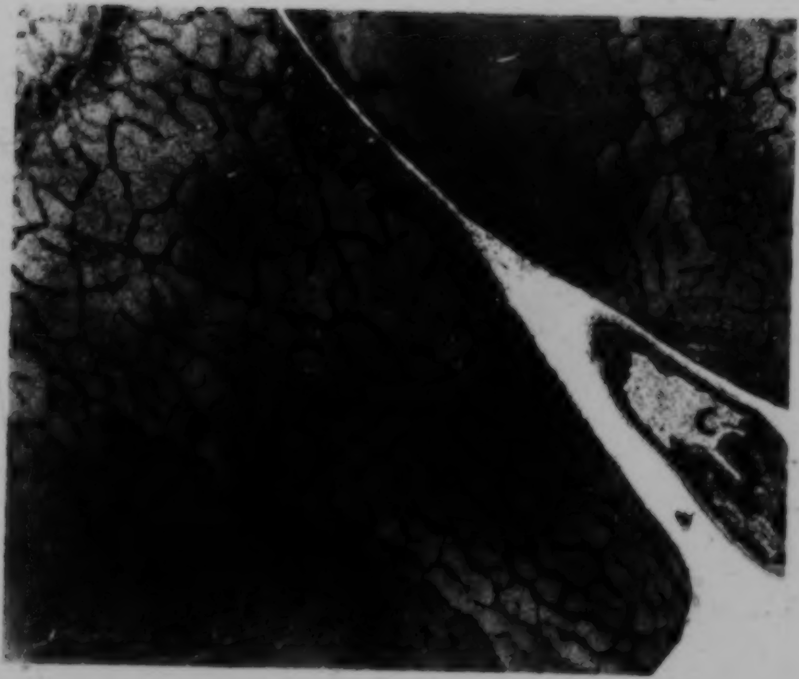


Plate 6

Transverse section, I-band superficial fibers. Lumbricals fixed by carpal tunnel perfusion. Note excellent preservation of mitochondria which surround myofibrils (x43,500).

Plate 7

Longitudinal section of a cell from the center of a lumbrical muscle fixed by carpal tunnel perfusion. Mitochondria (M) are swollen and disrupted. There is marked separation of myofibrils (MF) and swelling of Sr. All indicate poor preservation and cell swelling (x12,000).

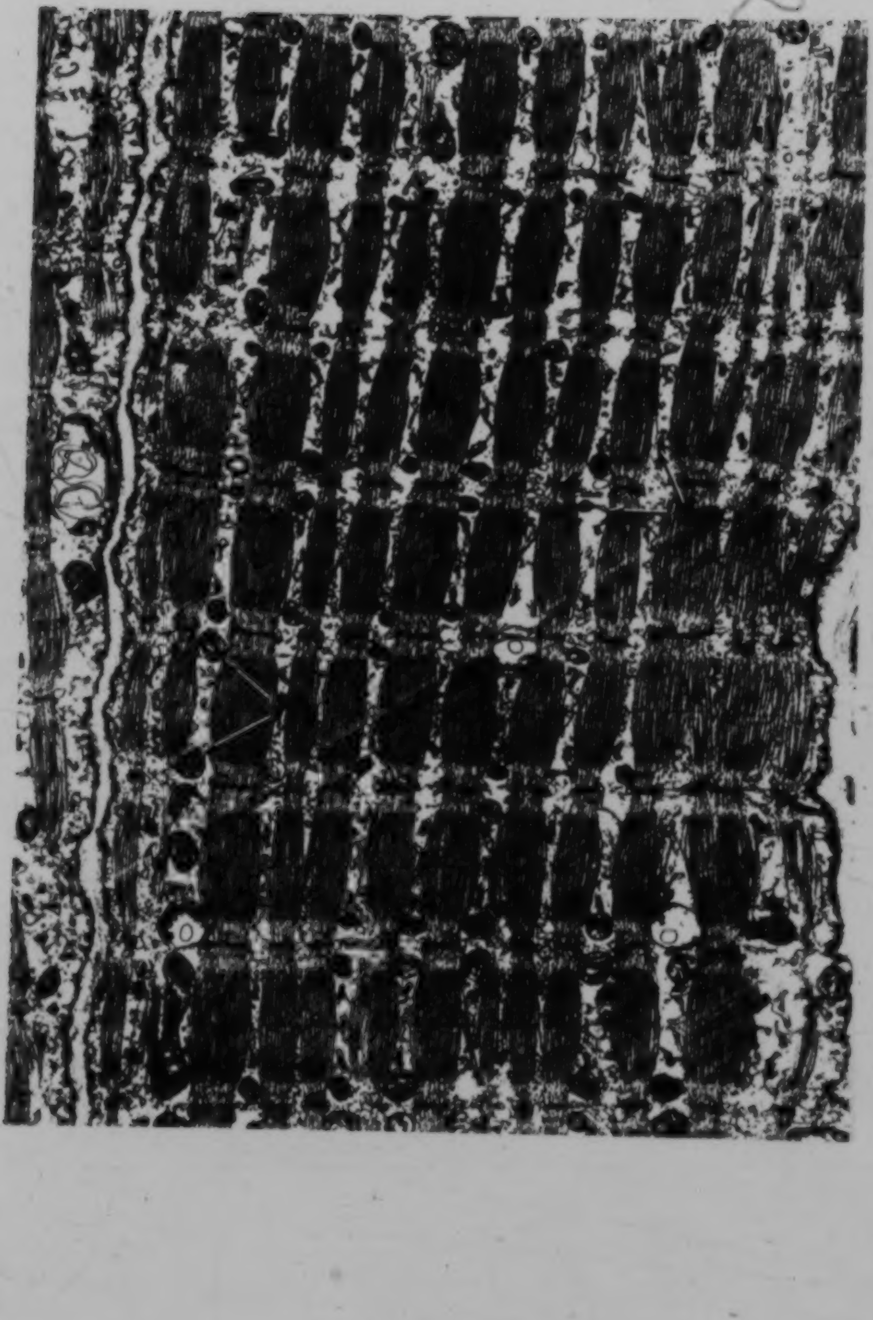


Plate 8

Longitudinal section of lumbrical muscle fixed by arterial perfusion of 1% glutaraldehyde. There is slight myofibrillar separation and mitochondrial (M) swelling but not as extensive as seen in adductor muscle fixed in a similar fashion (Plate 9). (x12,000)

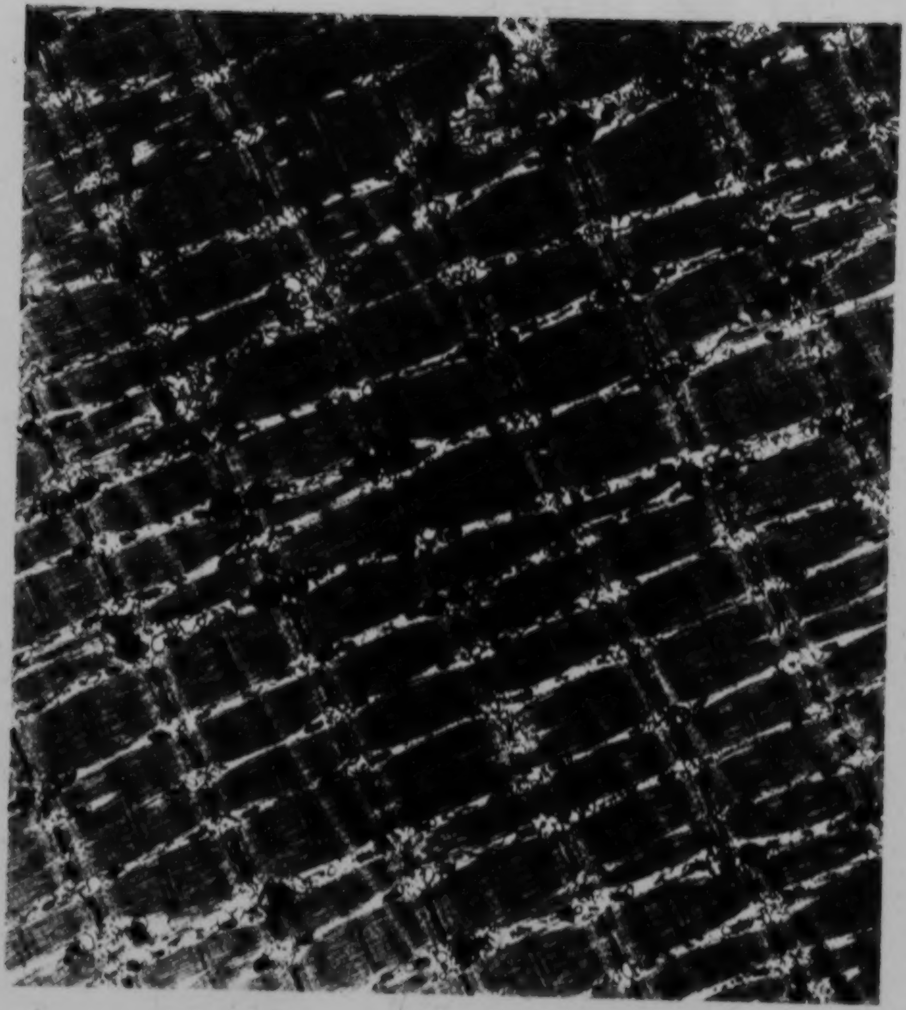


Plate 9

Adductor muscle; longitudinal section; 1% glutaraldehyde. There is considerable swelling with myofibrillar separation, slight extraction of mitochondria (M) and swelling of Sr. (x12,000)

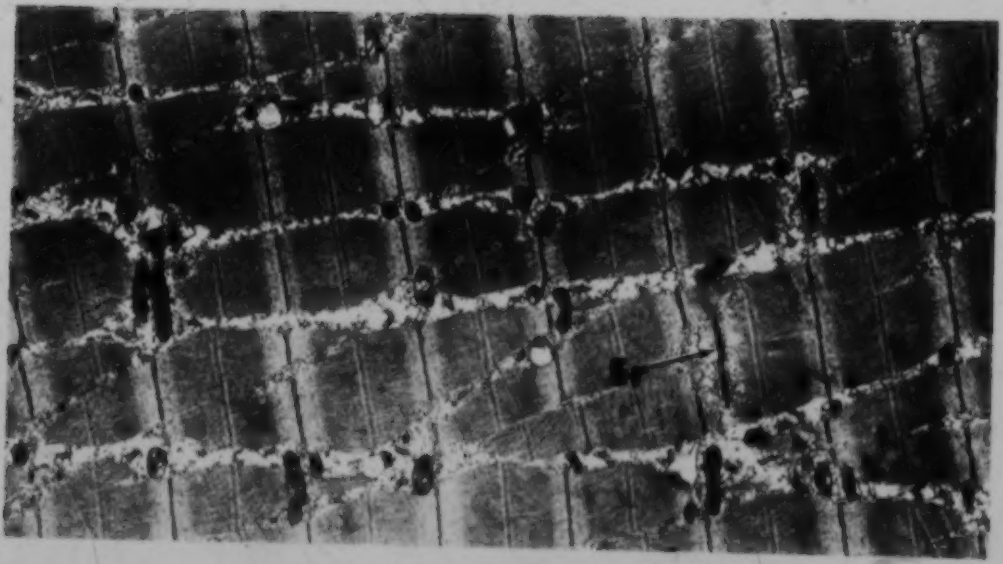


Plate 10

Lumbricals: 2.5% glutaraldehyde, immersion fixation. Cell organelles are well preserved. (x12,000)

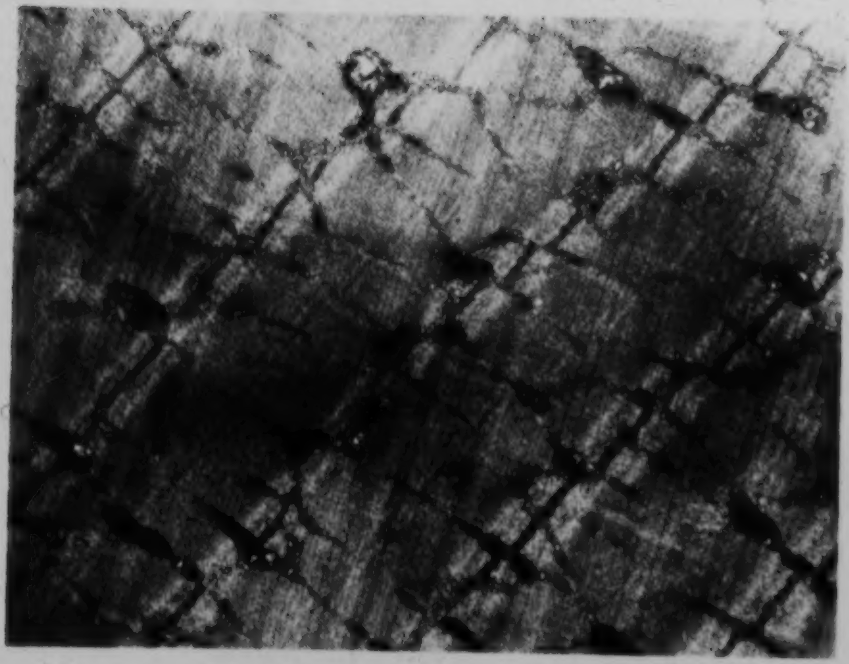


Plate 11

Adductor muscle: 2.5% glutaraldehyde immersion fixation. Organelles are well preserved. (x12,000)

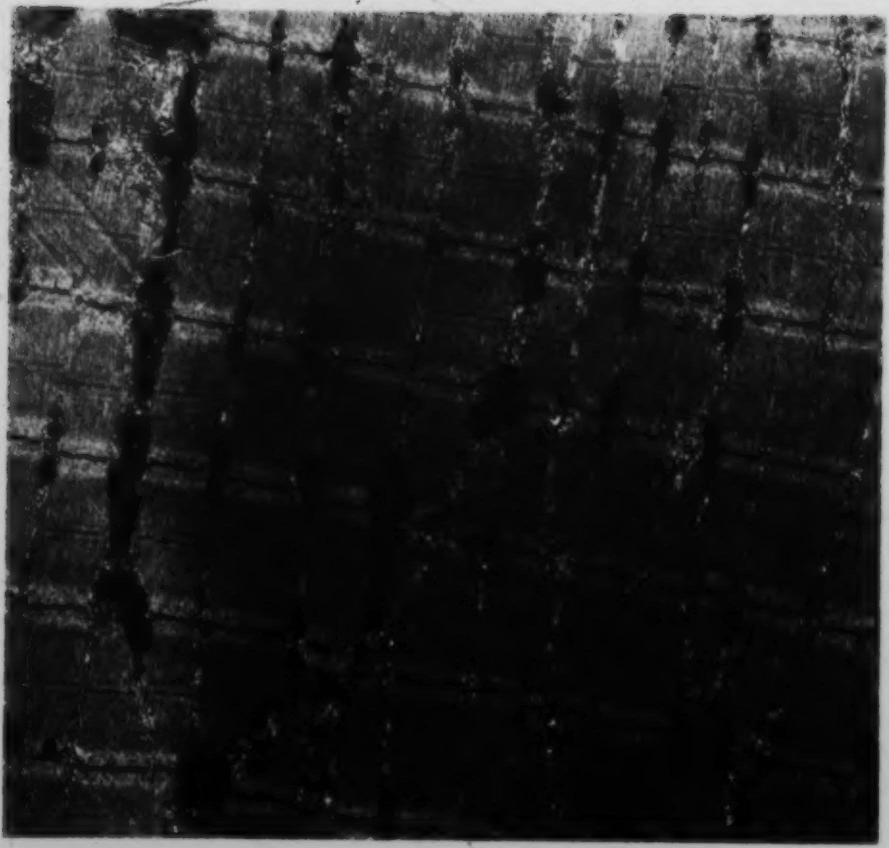


Plate 12

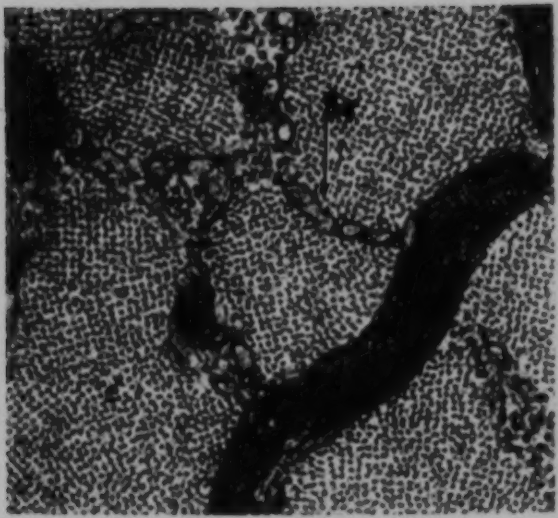
Lumbrical muscle fixed by arterial perfusion of 2.5% glutaraldehyde. Mitochondria have normal structure. Triads (T) are clearly visible. (x12,000)

Plate 13

Adductor muscle fixed by arterial perfusion of 2.5% glutarladehyde. There is excellent preservation of the cell and its organelles. (x12,000)

Plate 14

Transverse section; lumbrical muscle; I-band. Note direct continuity (short arrow) between Sr and mitochondrion (M). (x60,000)



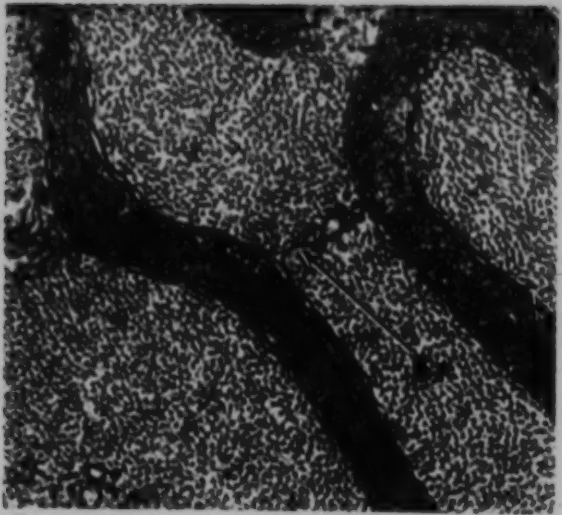


Plate 15

Note Sr like structure (Sr) connecting two mitochondria (M). Intermembranous compartments of the mitochondria appear continuous. (x60,000)

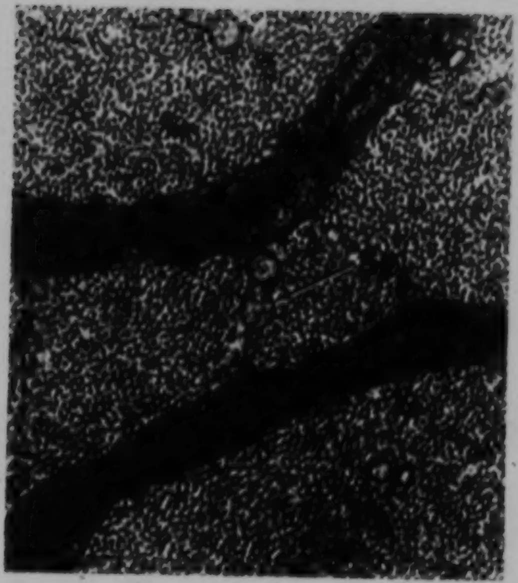


Plate 16

Mitochondria are connected by a short tubular connection. Such a short connection may also represent a section in an angle of a single mitochondrion. (x114,000)

Plate 17

Note the close association between a mitochondrion (M) and a terminal cisternae of the SR (TC). Superimposition of the two organelles may explain the appearance of direct continuity between the two organelles. The sine-wave structure (T) within the terminal cisternae may represent the foot processes adjacent to a T-tubule as viewed from within the terminal cisternae. (x159,000)

Plate 18

Apparent continuity between a sub-sarcolemmal component of SR (Sr) and the sarcolemma (S). Superimposition of sarcolemma and SR membrane during photography could also explain the appearance of continuity of the sarcolemma and SR membrane. The sub-sarcolemmal component Sr is not a segment of t-tubule since the absence of thin filaments identifies the area as the M-line. It may indicate a longitudinal extension of the t-tubule described by Forsmann and Girardier (1970) but this is unlikely. (x159,000)

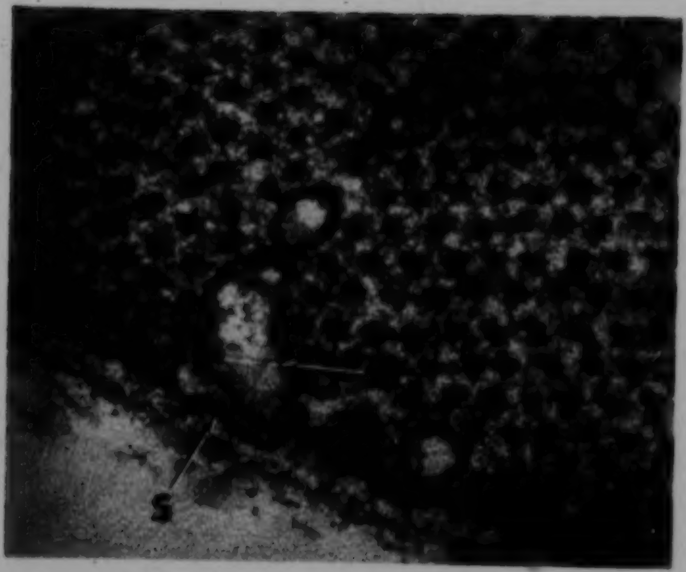

S

Plate 19

Mitochondrion, A-band. Note the pore-like communication (P) between the intermembranous space of the mitochondrion (M) and the sarcolemma (S) indicating direct continuity of the mitochondrion and the extracellular space. The diameter of the pore measures 40 nm. (x176,000)

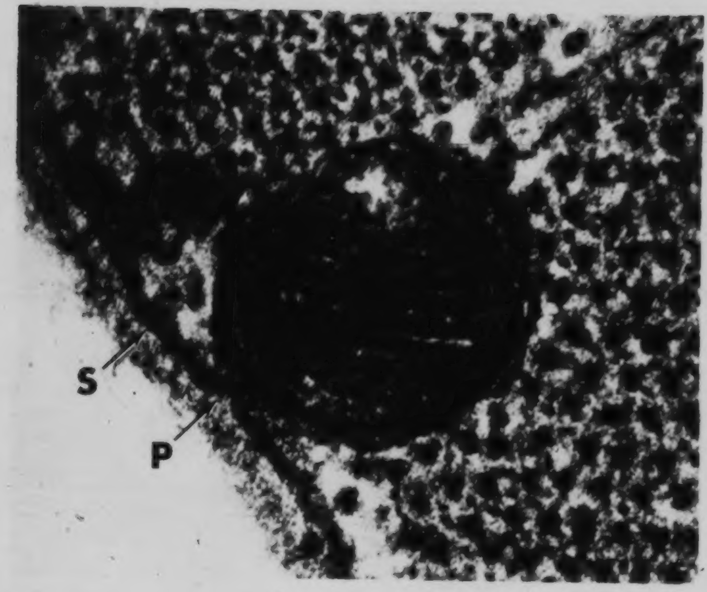

S
P

Plate 20

Note damaged mitochondrion (M) protruding through the sarcolemma (S). Other mitochondria (m) are normal. Therefore it is unlikely, but still possible, that this is an artifact of fixation. Extrusion of a degenerating mitochondrion by the cell is also possible. (x87,000)

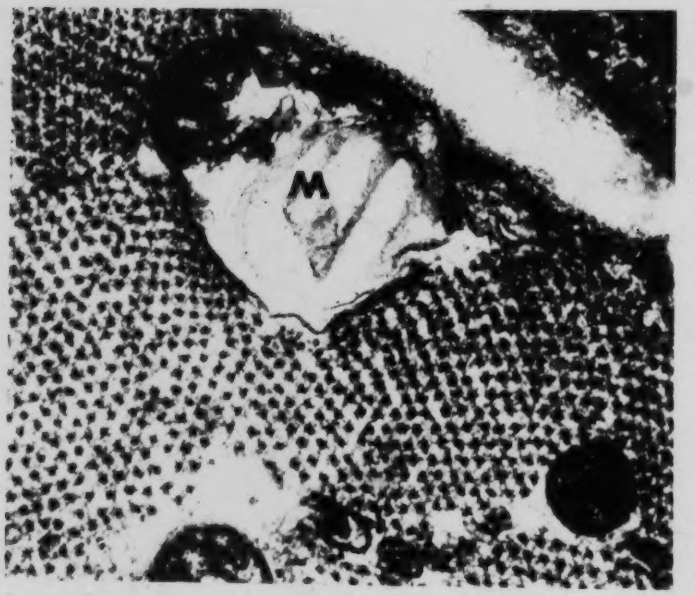

M

Plate 21

Round capillary containing a red blood cell in its lumen. Numerous vesicles are present in the endothelial cytoplasm. Note the extension of cytoplasm (E) into the lumen. I - interendothelial cleft. (x23,000)

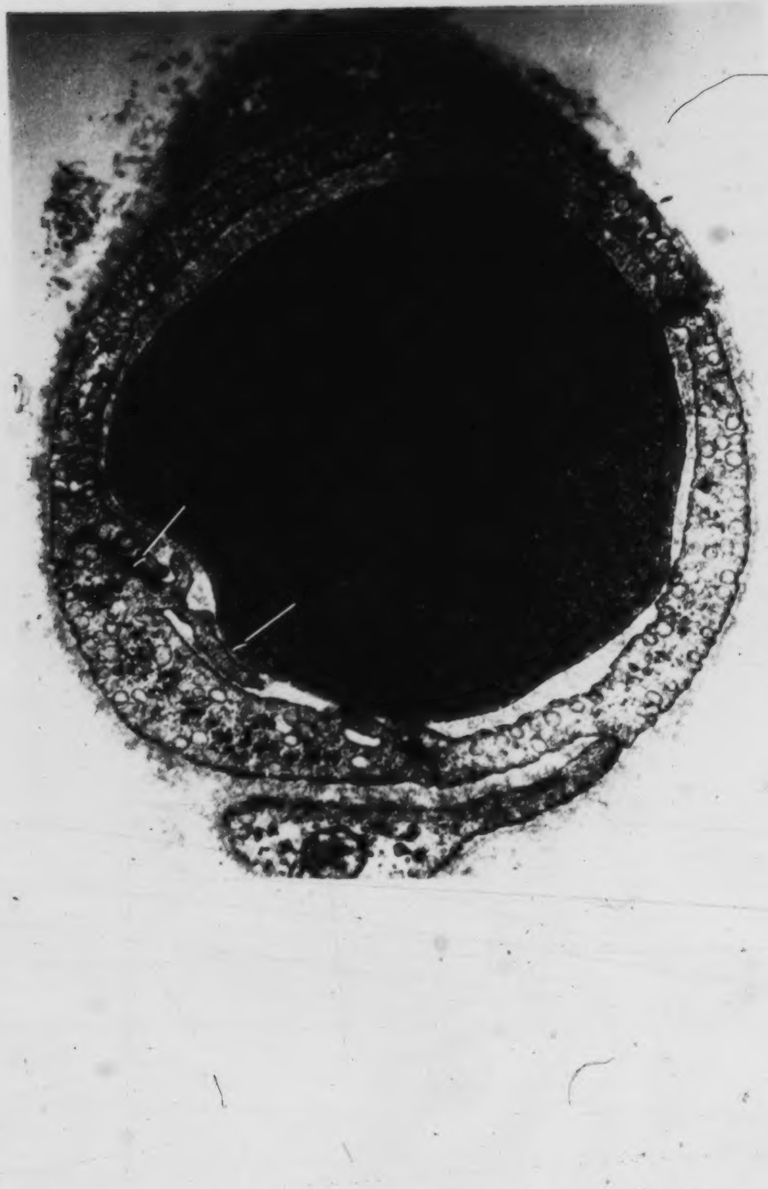


Plate 32

Collapsed capillary. Interendothelial cleft (I) appears flattened. (x16,000)

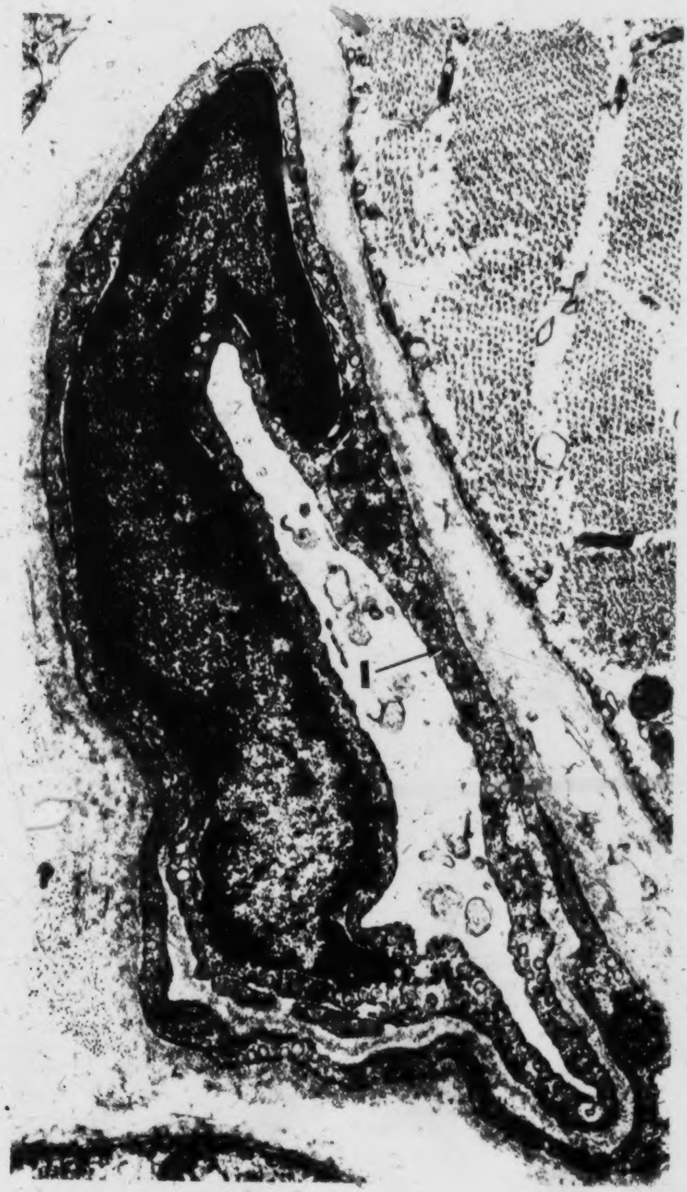

I

Plate 23

Partially collapsed capillary. (x16,000)

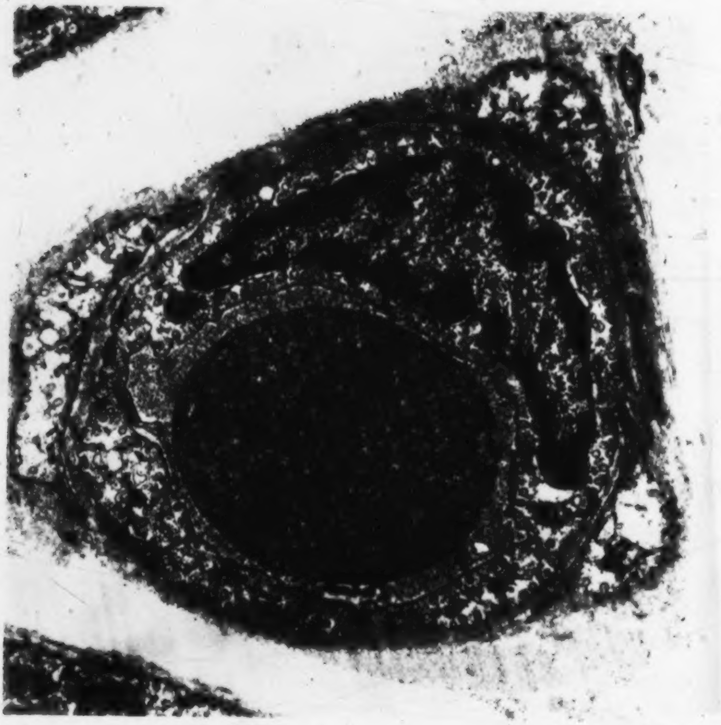


Plate 24

Partially collapsed capillary. Note several extensions (E) of endothelial cytoplasm projecting into the capillary lumen. These extensions occur at or adjacent to the interendothelial clefts. (x27,000)

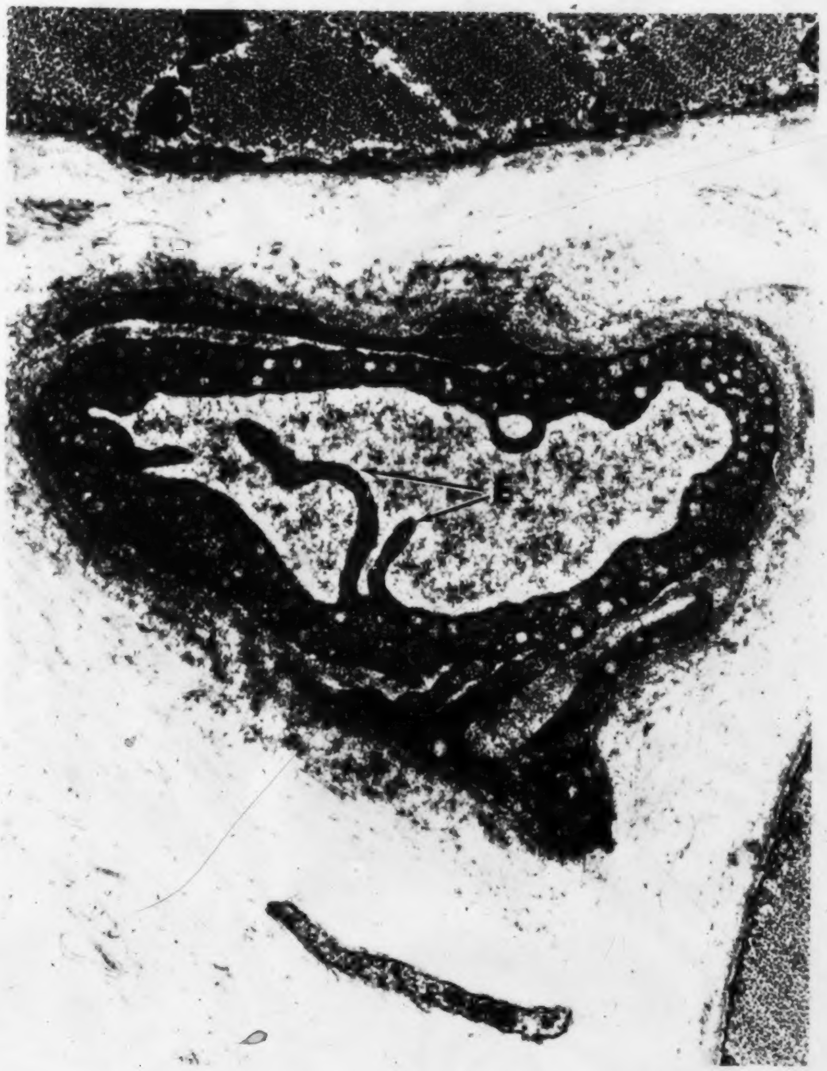

E

Plate 25

Note large vesicles (V), one on a cytoplasmic stalk, projecting into the capillary lumen adjacent to the interendothelial cleft (I). (x44,000)

Plate 26

Collapsed capillary in muscle at 80 mm Hg mean arterial pressure. An intraluminal extension of endothelium (E) is again evident. (x23,000)

Plate 27

Capillary in muscle with mean arterial pressure at 45 mm Hg. The endothelial vesicles appear smaller and less frequent. (x16,000)

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